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THE EFFECT OF ECLAMPTIC BLOOD UPON THE URINARY OUTPUT AND BLOOD PRESSURE OF HUMAN RECIPIENTS

By ERNEST W PAGE

(From the Department of Obstetrics and Gynecology University of Southern California School of Medicine Los Angeles)

he experiments described below were under- n with two purposes in mind, first, to deter- ̞ whether the transfer of 400 cc of blood ᴀ patients with severe preeclampsia or eclamp to normal pregnant women would have any ːt upon the blood pressure, urinary output, symptomatology of the recipient, and sec- ly, by standardization of the procedure, to over in eclamptic blood traces of postpituitary none in excess of the amount which might be ̜ent in normal blood

LITERATURE

ιe toxicity of the blood serum in eclampsia has been ed by numerous workers with varying results Be- n 1890 and 1895, all workers agreed that eclamptic I serum was more toxic to animals than normal n. The work of Volhard (1) and Schumacher (2) ̣ot support these conclusions. In 1920 Bumm (3) sferred blood from eclamptics to normal humans in ints up to 1 000 cc. "without demonstrating eclamptic ᴗns or cerebral intoxication" in the recipients The ιls are very meager and he does not mention any rvations on the blood pressure or urinary output of ̤ recipients. Lévy Solal and Tzank (4) believed that experimental intracardiac injection of serum from nptic patients showed a greater toxicity than that ᴉ normal pregnancy Lash and Welker (5) review literature and conclude from the study of two nptics that the blood serum proteins of normal and nptic women s blood show no evidence of toxicity ᴈ injection of large doses intraperitoneally into mice. ᴉ 1918 Hofbauer (6) advanced the theory that nptsia is due to an excessive secretion of postpituι hormone. In 1931 Anselmino and Hoffmann (7) sed considerable interest with their announcement ultrafiltrates from the blood of women with tox ̣s of late pregnancy inhibited diuresis in rabbits in ̨ast with control experiments. The rabbit, unfor ̦ly is not an ideal animal for assaying antidiuretic ̨ances but their results suggested that there was a ᴐnstrable excess of postpituitary hormone in the d of women with eclampsia or preeclampsia toxemias. 1934 with improved technique, Byrom and Wilson and Theobald (9) repeated these experiments in ndently and obtained negative results The first ᴈrs used the method of Burn (duration of inhibi

tion of diuresis of rats), while Theobald used dogs as test animals. The latter also determined that dosages of postpituitary hormone as low as 0 005 to 0 01 inter national units would inhibit diuresis in man, and in pregnant women near term. deWesselow and Griffiths (10) and Page (11) have failed to find any increased amount of pressor substances in the blood of patients with toxemias of pregnancy Hurwitz and Bullock (12) repeated the experiments of Anselmino and Hoffmann and found neither antidiuretic nor pressor substances in toxemic blood. Levitt (13) and Melville (14) inves tigated the problem with improved methods of extracting antidiuretic substances from blood and agree that their results cannot support the original contentions of Ansel mino and Hoffmann.

The normal mechanism of water and saline diuresis and its inhibition is well summarized by Best and Taylor (15) The exact mechanism of the postpituitary anti diuresis however is not understood. More recent in vestigations indicate that the hormone acts directly on the kidney to allow a greater reabsorption of chlorides and water by the tubules Renal denervation does not abolish the response (Samaan (16)) It is definitely established that several hundred times the minimal anti diuretic dosage is necessary to effect the blood pressure of unanesthetized animals, and since no antidiuretic sub stances have been found it is illogical to assume that the hypertension of eclampsia could be due to post pituitary hormone alone. The investigations of Heller and Urban (17) Levitt (13) Melville (14) and others show that the hormone disappears rapidly from the cir culating blood after injection and more slowly from human blood *in vitro* It has been demonstrated that the hormone is rapidly adsorbed in varying amounts by different body tissues liver kidney and brain tissue adsorbing far greater quantities than blood or any other tissue. There is a fairly rapid excretion of the hormone in the urine, and Heller and Urban suggest that there is also a specific (?) ferment responsible for its de struction. All of these factors may contribute to its rapid disappearance from the circulation. Following the injection of large amounts of pituitrin into animals, from 1/100 to 1/10,000 of the hypothetical amount present in a measured volume of blood may be recovered after 5 to 10 minutes (the amount depending largely on the method of extraction) while after 30 minutes no traces of the hormone can be discovered

Dock and Rytand (18) Collins and Hoffbauer (19), and others have failed to find pressor substances in the blood of animals with experimental renal hypertension,

rp (29), and Prizmetal Friedman and
1) have transfused normal humans with
...en s with essential and malignant hyper-
-t observing a rise of blood pressure in
Since most hypertensive states have many
...m n, these observations are of some im-
te study of eclampsia.

ICAL MATERIAL AND METHODS

to detect the presence of antidiuretic
an adequate diuresis must be estab-
is was done by two methods, admin-
constant volume (300 to 500 cc) of
mouth each hour and waiting for a
int high output of urine, and secondly,
a very large quantity of fluids by
intravenously at one time to establish
curve With the constant volume
immediate drop in volume during the
ing the injection of a substance, with
tory rise during the second or third
nterpreted as indicating the presence
tic substance With the diuretic curve
nch appears to be more sensitive, the
y solution or blood was given while
output was rapidly increasing A sud-
e, with interruption of the curve, in-
antidiuretic response In every pa-
d, a diuresis was established by one
o methods

es of observation were made

quantities of postpituitary solution
ed to determine the minimal dosage
d interrupt the human diuretic curve
iurdred cc of citrated blood from nor-
... cre administered to study the effects
transfusions on the urinary output,
o discover whether there is any anti-
rance present in that amount of nor-
L'ood

quantities of postpituitary solution
... with 500 cc of normal citrated blood,
' o .tard at body temperature for
... ots of time to determine whether

mone could be passed through the donor and
exert its antidiuretic effect upon the recipient

5 Varying amounts of blood were transferred
from patients with severe preeclampsia or eclamp
sia to women who were normal except for sec-
ondary anemia The effects of these transfusions
on the blood pressure, symptomatology, and di-
uretic curves of the recipients were studied

All of the recipients were women who were bed pa
tients on obstetrical or gynecological services The num
ber of experiments was limited because of strict criter
for the recipients which included (1) absence of ar
acute anemia with loss of blood volume or shock c
hypotension, (2) good general physical condition, (3) r
abnormal urinary findings, (4) normal circulatory sy
tem (5) no dehydration, (6) a definite indication fc
therapeutic transfusion, and (7) consent of the patien
Several experiments were discarded because a satis
factory diuresis could not be established, and sever
others because of technical difficulties (loss of a urin
specimen due to spill, delay in completion of transfusio
because of clotting of the blood, failure to keep th
needle in the vein, etc)

In each patient, a retention catheter was inserted an
left in for the duration of the experiment With th
constant volume method, specimens were collected eac
hour, or every thirty minutes With the diuretic curv
method, specimens were collected every ten minute
(leaving the catheter open), or the rate of output wa
recorded on a kymograph With the kymographic re
cordings a large cork was floated in a cylinder of suc
diameter that the addition of 10 cc of urine resulte
in a change of 1 mm on the record The disadvantag
of this method, however, was that the specific gravity
could not be determined on the individual specimens
and it was found to be more satisfactory if the observe
sat by the bedside, collected the samples every ten o
twenty minutes, measured the volume in a graduate
cylinder and recorded the specific gravity with a hy
drometer, supervised the fluid intake and administration
of the blood or postpituitary solution, and recorded th
blood pressure at frequent intervals Urinary output i
expressed as a rate in cubic centimeters per hour

When it was desired to establish a diuretic curve th
patient was given 500 cc of water to drink within 1!
minutes, and 800 cc of normal saline intravenously a
a very slow rate No harmful effects were observe
from this procedure alone, and there was no significan
rise in blood pressure Diuresis would beg~ within 3(
minutes

Obstetrical pituitrin (P D) containing 10 intern^{at}\{
units per cc was used exclusively In determining th

:d, and from the amount given the dosage of ry extract could be calculated As noted be s found that as little as 0 01 unit would exert able antidiuretic effect.

he pitultrin was given to the donors, it was to the buttocks about one minute before start- thdrawal of blood It would take between 12 inutes to withdraw 500 cc. The blood was 1 300 cc. of saline containing sufficient sodium prevent coagulation. When citrated blood was the juice of a lemon was given by mouth so liuretic action from the equivalent amount of ild be controlled The amount in either case y too small to exert any significant effect ases where blood was transferred from toxem rmals an effort was made to withdraw the ng an acute phase of the disease, particularly blood pressure was high. In all instances the started into the recipients veins within 10 ter its withdrawal and was given at a rapid o 60 cc. per minute) The blood pressure of :nt had previously been checked every few)r an hour to establish a basal level. In five periments where normal blood was given, it that in the absence of shock with hypotension irrhage, the systolic pressure did not rise more m., and there was no change in the diastolic except for a slight fall in two cases) when re given at the same rapid rate.

RESULTS

-seven determinations of antidiuretic ef- ether with blood pressure curves were

performed on 28 patients The data are not suit- able for presentation in tabular form, but are summarized below The more significant results are shown graphically in the accompanying charts The blood pressure curves are omitted in all but the last group of experiments, for there were no significant changes in any of them. The dosages of postpituitary hormone used were considerably smaller than the minimal dosage required to af- fect the blood pressure It was for this reason that the antidiuretic effect of the hormone was chosen to demonstrate the small traces which might be present in human blood

The two forms of charts presented may appear con fusing When the hourly urinary output is indicated by solid blocks, it indicates that the constant fluid intake method was used and the hourly intake is shown by unshaded blocks. Where kymographic recordings were made, or where the diuretic curve method was used, the rate of urine flow (cc. per hour) is shown by a simple line graph

1 The antidiuretic effects of postpituitary hor- mone Twelve determinations were made, us- ing various dosages of the hormone. Four char- acteristic responses are shown in Figure 1 An injection of 0 001 unit intramuscularly produced a questionable decrease in the urinary output in one experiment However, 0 01 unit produced a
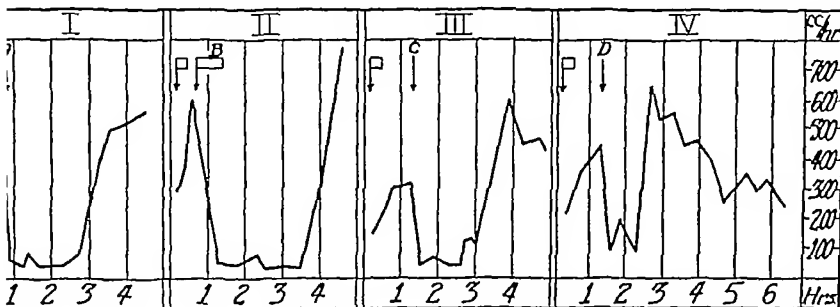
FFECT OF SMALL QUANTITIES OF POSTPITUITARY HORMONE UPON THE DIURESIS CURVES OF FOUR HUMAN SUBJECTS

curves obtained by ingestion of 500 cc. of fluids, with addition of 800 cc. of normal saline intravenously)

A 1 0 unit pituitrin intravenously
B 0 1 unit pituitrin in 400 cc. normal saline intravenously
C 1 0 unit pituitrin intramuscularly
D 0 01 unit pituitrin intravenously
⬜ = time fluids given by mouth and intravenously

FIG 2. EFFECT OF 500 CC. OF NORMAL BLOOD UPON THE URINARY OUTPUT

(In Subjects I, II and IV, diuresis was obtained by ingestion of 350 cc. of water every hour In Subject III, 800 cc. of saline were given intravenously at beginning of experiment.)

▮ BL. = 500 cc. citrated normal blood intravenously

In II and IV, there appears to be a definite but slight immediate antidiuretic action. In I and III there is a questionable delayed response.

pituitrin in 400 cc of citrated blood on the same patient The antidiuretic effects are almost identical

4   *The effects of in vivo mixtures of blood and postpituitary extract*   There have been only four opportunities for such experiments, and the results are equivocal   Twenty units of pituitrin were given intramuscularly to three male donors at the start of withdrawing the blood   In a fourth experiment, 40 units were given   The height and weight of the donors were obtained and the blood volume estimated   Five hundred cc of blood were withdrawn during the 15 minute interval following the injection, and given to normal anemic recipients   No immediate antidiuretic effects were noted   In one case there was a marked increase of urinary output, in two others essentially no change except for a slight decrease during the second hour, and in the last experiment there was a slight decrease within the

FIG. 3   EFFECT OF ADDING PITUITRIN TO BLOOD in vitro
I and II same patient two days between experiments.

▢ = 800 cc. normal saline intravenously
A   500 cc. normal blood intravenously
B   500 cc. normal blood to which had been added 2.0 units of pituitrin one hour before.

definite antidiuresis lasting for 45 minutes (Figure 1, IV) In two experiments, 0 1 unit produced a marked effect lasting from 1½ to 2½ hours (when given intravenously) In three experiments, 1 0 unit produced an antidiuretic response lasting from 1 to 3 hours, 0 5 unit produced the same result Two units were used twice with similar results, the effect lasting about two hours, 3 0 units were used twice, with immediate and marked antidiuretic action lasting over two hours

In all cases where amounts down to and including 0 1 unit of pituitrin were used, the rate of urinary flow fell from several hundred cc per hour to a rate well below 100 cc per hour, and in some instances there would be only 3 to 4 cc of urine secreted during a ten-minute interval It may be stated with some assurance, then, that any amount of postpituitary solution equivalent to 0 1 unit (and probably as low as 0 01 unit) may be readily detected by these methods

It would be worth while to mention the details of one case where symptoms developed from the retention of water resulting from antidiuresis Following the administration of 800 cc of saline intravenously together with 1 0 unit (0 1 cc of pituitrin) and 500 cc of water by mouth, there was an immediate drop in the rate of urinary output from 600 cc per hour to 20 cc per hour, the antidiuresis lasting for two hours At the end of this time, she began to complain of severe frontal and occipital headaches blurring of vision and she then developed homonymous hemianopsia She described scotomata which were apparently the same as those experienced by patients with late toxemias of pregnancy Within 30 minutes there was a marked increase in the urinary output with a compensatory rise to a maximum rate of 1200 cc per hour and all the symptoms disappeared At no time was there any rise in blood pressure The fundi were examined during the symptoms, but no retinal edema was noted

The absorption of postpituitary hormone by brain and the retention of water thereby is greater than that of any other tissue in the body (17) It would seem reasonable therefore that the symptoms in this case are due to cerebral edema This does not mean, however, that the visual disturbances and headaches experienced by patients with toxemias of pregnancy are due to the same causes, water intoxication alone others it may be that cerebral edema may produce similar symptoms

mal donors to patients with chronic secondary anemia were observed on 9 patients There was no significant rise of blood pressure during the transfusions, except for a brief rise of 10 to 15 mm Hg systolic during and immediately after the insertion of the needle This was interpreted as a pain response

Half of the amount of blood given was arbitrarily selected as representing fluid intake during that hour I have not been able to ascertain whether any or all of the blood should be counted as representing fluid intake The blood, however, does not appear to increase the total urinary output during the next few hours On the contrary, there is a constant decrease of urinary output during the *second* hour after the transfusion in all 9 experiments, and I am at a loss to explain this phenomenon Could it be due to the addition of proteins which increase the osomotic pressure of the total blood stream? Since the effects of postpituitary extract are immediate, this delayed response can hardly be attributed to the hormone.

In four of the nine experiments, however, there appeared to be a slight *immediate* drop in urinary output (e g, Figure 2, III and IV) This may be due to the presence of small traces (less than 0 01 oxytocic unit) of pituitrin in the 500 cc of normal blood Such findings are of significance as controls for the last group of experiments

3 The effects of in vitro mixtures of blood and postpituitary extract In six experiments, various amounts of postpituitary extract (0 12, 1 0, 1 4, 2 0, 2 0, and 6 0 units) were added to 500 cc of normal blood and allowed to stand for 30 to 60 minutes at body temperature before giving it to the recipient In each case, an immediate antidiuretic effect was noted after starting the transfusion The magnitude and duration of the effect was roughly equivalent to the amount of pituitrin added Under these conditions, therefore, blood does not completely destroy or inhibit the action of the hormone within the stated time limits

Figure 3 compares the effect of normal blood with blood to which had been added 2 0 units of pituitrin The same patient was utilized for
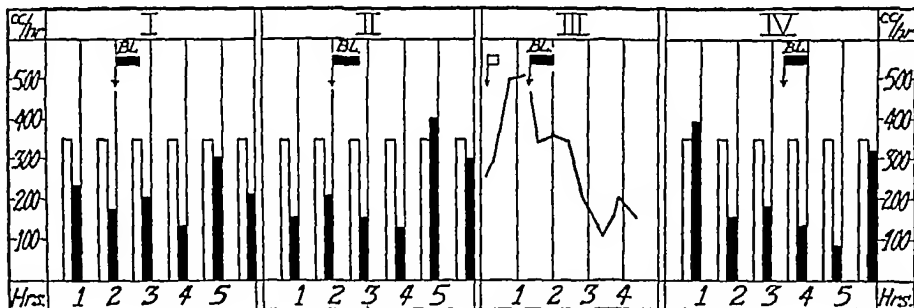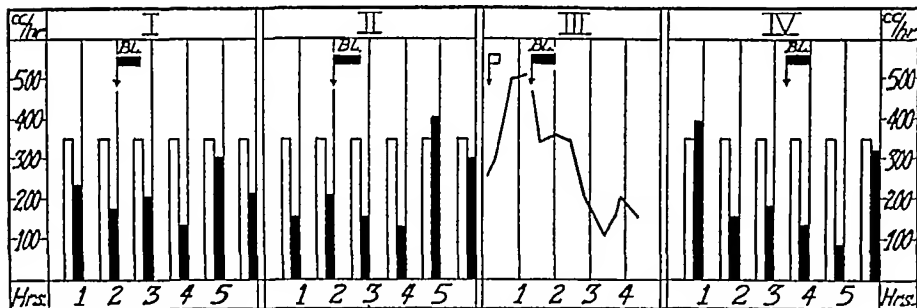
FIG. 2. EFFECT OF 500 CC. OF NORMAL BLOOD UPON THE URINARY OUTPUT

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▬▬▬ = 500 cc. citrated normal blood intravenously
↓ BL.

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height and weight of the donors were obtained and the blood volume estimated   Five hundred cc. of blood were withdrawn during the 15-minute interval following the injection, and given to normal anemic recipients   No immediate antidiuretic effects were noted. In one case there was a marked increase of urinary output, in two others essentially no change except for a slight decrease during the second hour, and in the last experiment there was a slight decrease within the

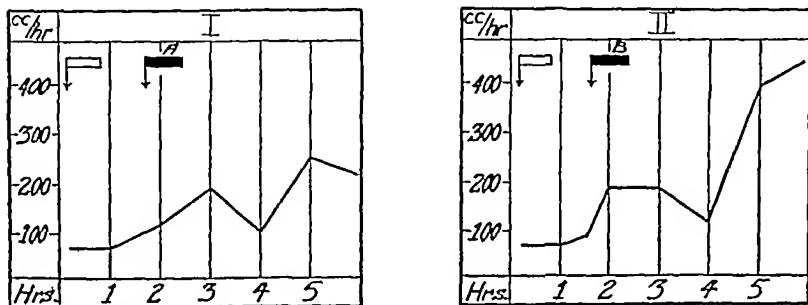FIG. 3   EFFECT OF ADDING PITUITRIN TO BLOOD *in vitro*
I and II same patient   two days between experiments.

☐ = 800 cc. normal saline intravenously
↓
A 500 cc. normal blood intravenously
B 500 cc. normal blood to which had been added 2.0 units of pituitrin one hour before.

Fig 4   Effect of Adding Pituitrin to Blood in vitro

A 1000 cc. fluids by mouth
B 200 cc. normal saline intravenously
C 400 cc. saline containing 0 12 unit pituitrin
D 400 cc. blood   0 12 unit pituitrin added to blood one hour before.
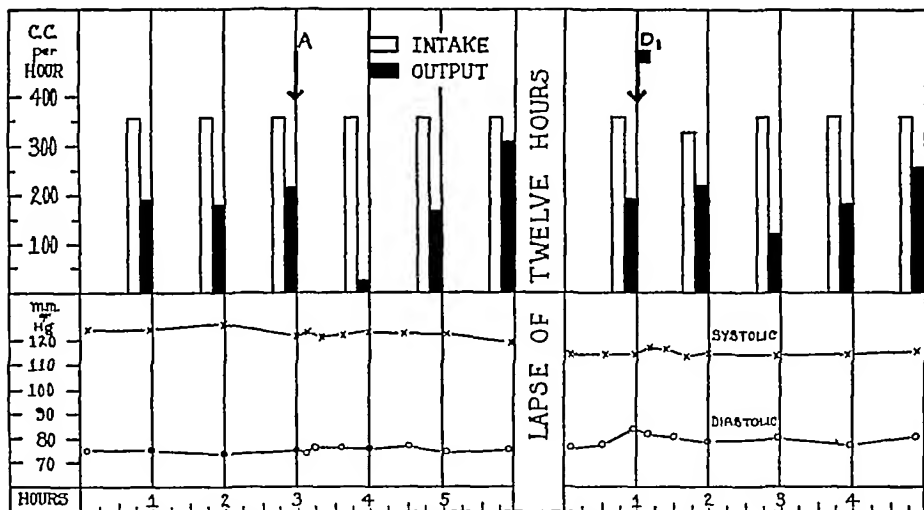
Fig 5   Effect of Blood from Severe Eclamptic

A 0 5 unit pituitrin intramuscularly (alone)

D₁. 400 cc. blood from Donor 1
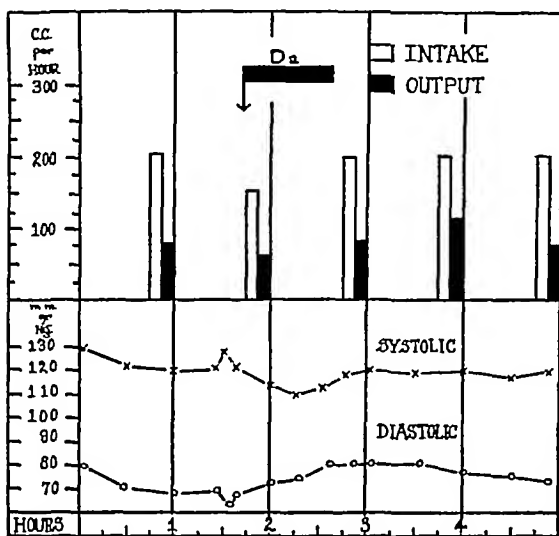
Fig 6   Effect of Blood from Mild Eclamptic

D₂. 400 cc. of blood from Donor 2.
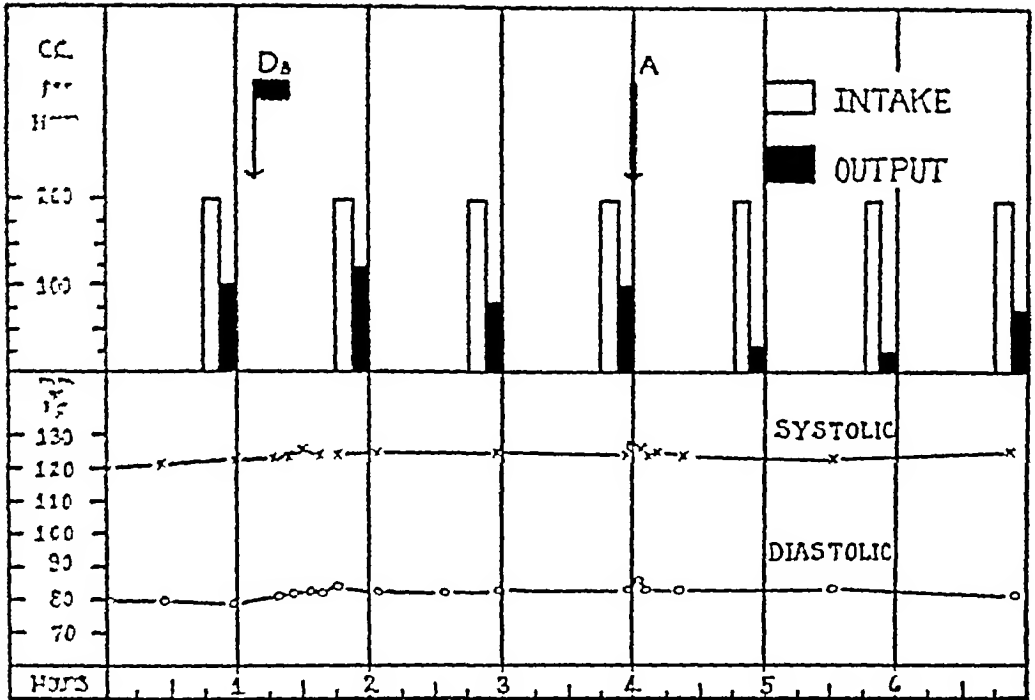
Fig 7   Effect of Blood from Severe Preeclampsia
D_3 400 cc. of blood from Donor 3
A 2 units pituitrin intramuscularly
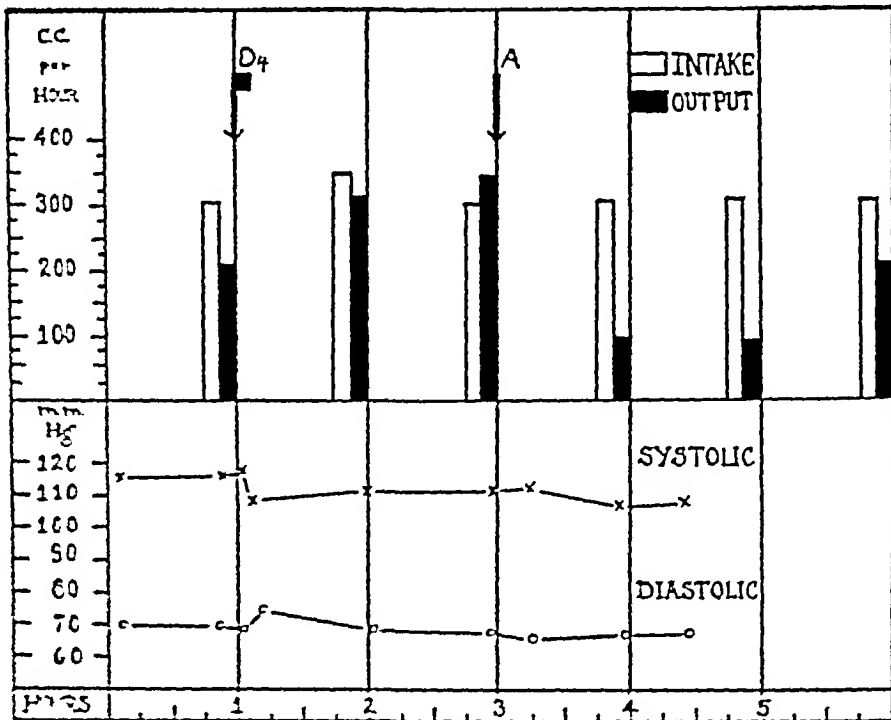
Fig 8   Effect of Blood from Severe Preeclampsia (and Probable
Preexisting Chronic Nephritis)
D_4 425 cc. of blood from Donor 4
A 2.0 units pituitrin intramuscularly

amounts present in normal blood, particularly if the donor is not hydrated, are probably demonstrable by the methods used, any increased quantity should be detectable

Small traces of postpituitary principle are capable of producing marked antidiuretic effects in humans   Even large dosages of the same substance rarely raise the blood pressure of normal unanesthetized humans   Since the pressor and antidiuretic substances are apparently inseparable, the failure to detect the antidiuretic principle in eclamptic blood either by extraction and assay on animals or, as in this instance, by direct transfusion to suitably prepared human recipients almost precludes the possibility that the hormone is responsible for the hypertension

While the number of such operations on human test subjects are necessarily limited, the results are consistent enough to conclude that very small traces of active postpituitary hormone may be detected by these methods, and that such traces are not present in eclamptic blood   The results, therefore, are not in agreement with the conclusions of Anselmino and Hoffmann, but confirm the results of animal experimentation by subsequent workers (8, 9, 12, 13, 14)

None of the work brought forward to date (including the present report) actually eliminates the possibility that the hypophysis is in some way involved in the production of the eclamptic syndrome   It is practically certain that the lesions of eclampsia are the result of either a physical or chemical alteration of the blood, and there is much evidence to show that there are endocrine disturbances associated with toxemias of pregnancy (summarized by Shute (22))   Whether the imbalance of sex hormones described in eclampsia is of primary or secondary importance is not known   With our present knowledge of pituitary interrelationships, it is inconceivable that there could be a marked endocrine disturbance without involvement of hypophyseal function   It is possible that the diminished urinary output in eclampsia may be partially or wholly due to a suppression of diuretic substances from the anterior pituitary   It is still possible that an excess of postpituitary hormone is so fixed or adsorbed by other body tissues that it cannot be demonstrated in the blood stream   It can merely

be said that the theories of an eclamptic "toxin" or of a hypersecretion of the postpituitary are still attractive, but that they have as yet no valid experimental proof

SUMMARY AND CONCLUSIONS

1 Amounts of pituitrin as low as 0 01 unit may be readily detected on suitably prepared human test subjects

2 Transfusions of 500 cc. of blood from normal donors does not result in a significantly altered blood pressure or urinary output in the recipient, although the results suggest the presence of traces of postpituitary hormone in normal blood.

3 If small amounts of pituitrin are added to human blood *in vitro* and allowed to stand for 30 to 60 minutes before transfusion, a definite antidiuretic response may be obtained in the recipient.

4 If large amounts of pituitrin are given to donors prior to the withdrawal of blood, no definite antidiuretic response can be obtained in the recipient, indicating a rapid elimination or destruction of the hormone from the circulating blood stream

5 When amounts of 400 cc. of blood are rapidly transferred from patients with eclampsia or severe preeclampsia to normal pregnant women, the recipients do not show any rise in blood pressure, interference with diuresis, or untoward symptoms

6 The results do not support the contention that there is a markedly toxic substance in toxemic blood, nor the theory that there is a hypersecretion of the postpituitary gland in eclampsia. No pressor substance has been demonstrated in eclamptic blood by these methods

APPENDIX

Following are the protocols of the cases where blood was transferred from toxemic donors to normal recipients

Donor 1   Caucasian female, age 29 gravida 1 para 0, two days from term, referred in by a private physician because of severe headaches for five days edema of the legs for two weeks nausea and vomiting for one day, and epigastric pain for one day   Blood pressure on admission was 200/106   The urine showed 6.1 grams of albumin per liter   At 2 00 p.m., the blood pressure was

210/120 - 1 at th < time 400 cc. of blood were with-
drawn and citrated When the needle was withdrawn
from the vein at 2 14 pm, she had her first convulsion
Th s was follo ed by a prolonged coma from which she
never fully reco ered The pulse rate remained between
140 and 160 The usual conservative regime was insti-
tuted and the membranes ruptured Progress in labor
was slow Cesarean section was proposed, but abandoned
because of her critical condition She was delivered of
a still born by low forceps at 7 pm the following day,
and died a few hours later At the time of delivery,
the pulse was 170, patient had been in a deep coma for
24 hours and the temperature was 103° F Autopsy was
refused *Diagnosis* Antepartum eclampsia, severe grade

Recipient 1 White female, age 21, gravida 1, para 0,
had been under observation for three days because of a
3½ mon hs pregnancy with incomplete abortion Gen-
eral condition was good, and all bleeding had ceased
Hemoglobin was 9.4 grams per 100 cc., red blood count
5.05 million Twelve hours before the transfusion was
given diuresis was established, and the effect of 0 5 unit
of postpituitary extract was determined There was an
immediate and marked antidiuresis as illustrated in the
first part of Figure 5 The specific gravity increased
from 1 004 to 1 011 With the compensatory increase in
urinary output two hours later, the specific gravity de-
creased to 1 001 There was no change in blood pressure

She received the blood from Donor 1 at 2 20 p.m the
follow ng day (six minutes after completing the removal
of the blood) The results are shown in the second
part of Figure 5 There was a very slight decrease in
urinary output the *second hour* after receiving the blood,
as was noted with normal transfusions The specific
gravity increased from 1 005 to 1 009 and returned to
1 04 with the last specimen There was no significant
change in blood pressure

In th s case each urine specimen before and after the
transfus on was examined microscopically No abnormal
elements were found prior to the transfusion Following
the transfus on, a few red blood cells (1 to 2 per high
power field in a centrifuged specimen) and some renal
epithelial cells appeared After three hours these dis-
appeared Since the sed ment was not studied with nor-
mal transf s ons no conclus ons are drawn

Recipient 1 had a d lat on and curettage the next
day and left the hosp al three days later n good con-

for study showed normal values for nonprotein
urea nitrogen, and CO combining power Th
was 5 1 mgm per cent A normal delivery of
7 ounce living infant occurred 10 hours h
were no further convulsions and the blood p
urine were normal at the time of discharge
amination six weeks and six months later
Intrapartum eclampsia, mild grade

Recipient 2 The blood removed was
transferred to a colored primipara of 19, 4
partum, with moderate secondary anemia res
a postpartum hemorrhage The hemoglobin
grams per 100 cc An interesting feature
servation was that the recipient had a mild
toxemia (blood pressure 150/100, 2 + albu
aches and edema) at the time of her delivery
symptoms had all subsided before the blood
Thus we are certain that this recipient was "
to toxemias"

The results of the transfusion are shown in
There was essentially no change in the urinary
specific gravity The slight rise of blood pres
start of the transfusion was coincident with th
of the needle No subjective symptoms deve
ing or after the transfusion

Donor 3 A Mexican female of 20 was ac
cause of severe toxic symptoms This was l
pregnancy She had had sepsis but no tox
her first pregnancy Past history otherwise w
nificant She was 8 months pregnant, and
of swelling of the face, trunk, hands and legs
weeks, marked visual disturbances with thre
when she could " see only the right half of a
a room," and severe headaches Examinatic
generalized edema, with pitting of the lower b
body The uterus was 50 cm. above the
tense, and no fetus could be palpated or fetal h
heard Blood pressure was 170/110 The
tained 4 17 grams of albumin per liter and sl
merous casts and pus cells, with occasional
cells Nonprotein nitrogen was 24, CO₂ combin
46, uric acid 4 4

Shortly after admission a retention cathete
serted and the hourly urinary output meas
output during the 8 hours preceding delivery
of a fluid intake of 1,200 cc. was onl, 197 cc
20-8-50-30 and 35 cc per respective hour) T

Recipient 3 The 400 cc. of blood removed from Donor 3 were immediately introduced into a 21 year old Caucasian primigravida who was in the hospital because of an incomplete abortion. Her general condition was good. Hemoglobin was 11.6 grams per 100 cc. The results of the experiment are shown in Figure 7 No antidiuretic effect o the donor's blood could be detected, *even though the donor herself was having a marked oliguria at the time the blood was taken* There was no pressor action from the transfusion and the recipient developed no symptoms during or after the transfusion. Note the marked antidiuretic effect of pituitrin (2 0 units) given 3 hours after the blood was given.

Donor 4 A 34 year old white primigravida entered the hospital 11 weeks from term complaining of severe headaches and visual disturbances for two weeks edema of the ankles for one month, and absence of fetal movements for two weeks. Blood pressure was 160/110 The urine contained 0.83 gram of albumin per liter with numerous casts and pus cells. Blood chemistry values were all within normal limits. Four hundred and twenty five cc of blood were removed shortly after admission, and citrated for transfusion. The following day she delivered a 3^r cm. macerated fetus and a placenta which was almost completely infarcted. The blood pressure fell to normal, but rose again to 170/110 on the 12th postpartum day falling to 150/90 on the 15th day *Diagnostic impression* Preeclamptic toxemia, severe grade, probably superimposed on a preexistent chronic nephritis

Recipient 4 The blood was immediately introduced at a rapid rate into a white female of 28 who had had a normal delivery of her third baby on the preceding day On admission, her hemoglobin was found to be 7 1 grams per 100 cc. and her red blood count 2.98 million, although otherwise the physical examination had not been remarkable. The results of the transfusion are shown in Figure 8. There was a slight drop in the blood pressure and a rise in the urinary output. Note the effect of 2 units (0.2 cc.) of pituitrin two hours after the transfusion. While receiving the blood the recipient developed numerous large urticarial wheals over the trunk, but there were no other toxic symptoms

(In addition to the experiments just reported, 500 cc. of blood were transferred from a 17 year old girl (non-pregnant) with malignant hypertension (blood pressure 280/150 amblyopia, uremia) to a woman who had just had a postpartum hemorrhage, but who had recovered from her shock. No antidiuretic or pressor response was noted in the recipient)

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STUDIES IN THE PHYSIOLOGY OF ARTIFICIAL FEVER. I
CHANGES IN THE BLOOD VOLUME AND WATER BALANCE

By JOHN G GIBSON, 2D,[1] AND ISRAEL KOPP [2]

(From the Department of Medicine Harvard University the Medical Clinic of the Peter Bent Brigham Hospital and the Department of Neurosyphilis of the Boston Psychopathic Hospital, Boston)

During recent years great interest has been aroused in the use of artificially induced fever in the treatment of disease   Numerous claims have been made for the benefits of fever therapy but very little attention has been paid to the changes it brings about in the coordinated functions of the body, well and diseased

The dangers of fever therapy have been emphasized by many authors   Reactions of varying severity from nausea and vomiting to tetany (1, 2, 3), delirious episodes (4), convulsions (5), heat stroke (3) and shock (2, 3, 6 7, 8), have been described and deaths have been reported (9, 10, 11, 12, 13, 14)

The purpose of the studies reported in this series of communications was to determine the changes resulting from artifically induced fever in circulating blood volume, water balance, acid-base equilibrium and hemodynamics and the relationship of these changes to the clinical condition of the patient   Our studies on the first two of these factors are reported herein

No thorough studies on changes in blood volume during artifical fever have been reported In the opinion of several fever therapists (15, 16 17) no significant alterations in circulating blood volume occur during artifical fever if water is given liberally, although some workers have reported that some degree of blood concentration does occur (18, 19, 20)   Hemoconcentration as evidenced by increases in red counts and hemoglobin values has been regarded by some as evidence of a reduction in volume (19 20), but others (15) interpret the increase in hemoglobin as evidence of a real increase in total hemoglobin to meet increased oxygen requirements   By means of the CO method (21) and a dye method

(22), decreases in plasma volume have been observed after artificially induced fever in animals

Reliable studies of the water balance in artificial fever were not found in the literature, although the high fluid requirement amounting to as much as 3 to 6 liters during a 5 to 6 hour treatment has been commented on (15, 23)

METHODS

The dye method of determining the plasma and total blood volume of Gibson and Evans (24) was used. In the experiments conducted in Rochester N Y and Dayton Ohio colorimetry was done by the technique described by Gibson and Evelyn (25) with the photoelectric colorimeter of Evelyn and Cipriani (26)   The " direct " method (24) measures the plasma and total blood volume obtaining at the time of dye injection the determination being based upon the dilution in the blood stream of a measured amount of Evans Blue of known concentration.   In the " indirect " method an initial determination of blood volume is made by the " direct " method on the afternoon of the day preceding the fever treatment (Figure 1)   On the morning of treatment the rate of disappearance of dye from the blood stream is determined from the dye concentration of blood serum samples taken at regular intervals over a two-hour period, and this rate is assumed to be constant throughout the ensuing experimental period   Changes in plasma volume are estimated from the deviation of the dye concentration of blood serum samples taken during fever from synchronous points on the disappearance slope, higher or lower density values representing a reduction from or increase over the prefebrile plasma volume respectively   At the end of treatment the plasma volume is again determined by the direct method, and the agreement between the final volume so determined and the final volume as estimated from the blood serum sample taken just prior to the final dye injection constitutes a check upon the accuracy with which changes in plasma volume during the fever period have been measured.

That the disappearance slope of Evans Blue does remain fairly constant during prolonged febrile periods is evidenced by the fact that in 20 of the 31 experiments reported herein conducted as described above the final estimated plasma volume was within plus or minus 3 per cent of the value obtained by actual redetermination. The narrow limits within which the final check fell in

[1] Research Fellow in Medicine, Harvard Medical School.

[2] Research Associate in the Massachusetts Department of Mental Diseases.

① INITIAL BLOOD VOLUME

4 20 P.M.
11 28-35
PLASMA 1770 cc
CELL 1345 cc
TOTAL BLOOD 3115 cc

⑥ FINAL BLOOD VOLUME

	ESTIMATED	DETERMINED
PLASMA	1575	1585
CELL	1285	1290
TOTAL BLOOD	2860	2875

CONCENTRATION OF DYE IN BLOOD SERUM

14 13 12 11 10 09

DYE DISAPPEARANCE SLOPE

8 10 A.M.
11 29 35

HOURS — 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

RECTAL TEMPERATURE °F — 107 105 103 101 99 97

② CONTROL PERIOD → INDUCTION ← ③ — FEVER PERIOD ④ — RECOVERY → ⑤

FLUID INTAKE AND OUTPUT — cc 300 200 100 0

TOTAL INTAKE 3550 cc
VOIDED 185 cc

PLASMA VOLUME — VOLUME IN cc 1800 1700 1600 1500
-1067

TOTAL BLOOD VOLUME — 3100 3000 2900 2800
-567

FIG 1   METHOD OF DETERMINING CHANGES IN BLOOD VOLUME DURING ARTIFICIAL FEVER

The initial blood volume (1) was determined the afternoon of the day preceding treatment The rate of disappearance of the dye from the blood stream was determined during a prefebrile control period (2) the day of treatment During induction of fever (3) the concentration of dye in the blood stream rose, indicating a reduction in the plasma and total blood volume During maintenance (4) a rise and later a fall in volume which continued through recovery (5) occurred The repeated plasma volume at the end of treatment (6) agreed well with the final estimated plasma volume

these cases was the justification for the modified procedure followed in Cases R-2 to R-7 inclusive and D-1 to D-3 inclusive, in which the disappearance slope was not determined prior to the induction of fever A single blood sample was taken before the patient was placed in the fever cabinet and the disappearance slope arbitrarily constructed in such a manner that the final plasma volume as estimated from the slope equalled the final redetermined plasma volume after the latter had been corrected for blood withdrawn in sampling

We have found slight degrees of icterus to occur during artificial fever, but no colorimetric error is introduced thereby since the absorption of serum pigments at the wavelength 620 millimicrons (at which measurements of Evans Blue are made) was found to be so low that slight increases thereof were negligible in relation to the

absorption due to the high concentrations of dye utilized in these plasma volume experiments

It is our opinion that, by the methods employed, changes in plasma volume were measured to within 3 per cent No claim for great accuracy is made for changes in red cell and total blood volume because of the differences known to exist in red cell content of peripheral and capillary blood, particularly when severe physiological disturbances are taking place

All patients were weighed nude both before and after treatment on lever type platform scales Weights measured in pounds were obtained to the nearest one-eighth pound, and those measured in kilograms to the nearest 0 05 kgm Thus, changes in weight could be considered in terms of water to within about 50 cc

Patients treated by typhoid vaccine received tap water

by mouth those treated by diathermy and hot moist circulating air received 0 6 per cent saline solution by mouth or 0 85 per cent saline intravenously—patients treated by radiant energy were given tap water by mouth and in addition NaCl in 2 per cent solution in amounts sufficient to make the salt content of the total fluid intake approximately equal to a 0.5 per cent saline solution. Fluids given by mouth were measured in graduates and those given intravenously from the usual graduated clysis flask. It is thought that total fluid intake during each treatment was measured with an accuracy of about 50 cc. Volume of urine and vomitus was measured in graduated cylinders

ANALYTIC CONSIDERATIONS

A bout of artificial fever may be thought of as consisting of three periods the period of induction of temperature to the desired level during which the mechanisms of the body for temperature regulation are overcome, the period of maintenance at the desired temperature, and the period of recovery to normal temperature, during which the heat regulating mechanisms are again permitted to function successfully Changes in plasma and total blood volume were analyzed in relation to these three periods

It was assumed that all water lost is withdrawn directly from the blood stream via the skin lungs or kidneys that weight changes may be considered in terms of water and that weight change represents the true difference between the gross water loss from the blood stream and total fluid intake, according to the equation

Gross Water Loss = Total Fluid Intake +
         Weight Loss or — Weight Gain   (1)

The amount of fluid withdrawn from tissue spaces in patients inadequately supplied with water or the amount entering the tissue spaces in those in whom excessive fluids were given was calculated by the equation

ing the short treatments to 500 cc. during the long treatments could not be included in total fluid intake. On the other hand it was not possible to determine, except in those cases in which all fluids were given intravenously that all fluids administered were completely absorbed by the end of the experimental period. These two sources of error tend to compensate each other It is felt that gross water loss and tissue fluid changes calculated in the above manner are valid to within about 5 per cent, and are of value for the clinical interpretation of the signs and symptoms presented during artificial fever

MATERIAL STUDIED

Thirty-one studies were carried out on 25 patients 21 of whom were males and 4 females All were undergoing fever therapy, the diagnoses being indicated in Table III In 3 studies fever was induced by the intravenous injection of killed typhoid organisms. In 8 studies diathermy was used, the apparatus employed being the Super Power" unit, with segmented electrodes encircling the arms thighs, and waist, the current varying from 1500 to 2600 milliamperes In one case treated with typhoid vaccine and in two cases treated by diathermy additional heating in the form of radiant energy (carbon filament lamp) was used. In 13 studies 3 of which were made at Dayton, Ohio fever was induced by hot moist circulating air in the ' Kettering Hypertherm (27) In the 10 studies made in Boston the relative humidity in the cabinet was maintained between 30 and 50 per cent during induction of fever Dry bulb temperature was about 155 to 160 F and the wet bulb about 130 to 135 F during induction During maintenance the air current was turned off and the patient was covered with blankets In the 3 cases studied in Dayton, relative humidity was about 80 per cent, dry bulb and wet bulb temperatures being about 130 F and 125 F during induction, and 125° F and 120 F during maintenance respectively Air speeds were somewhat

$$\text{Tissue Fluid}\quad\begin{matrix}\text{Loss} = \text{Gross Water Loss} &- \left(\text{Total Fluid Intake}\begin{array}{c}-\text{ Decrease in Plasma Volume}\\ \text{or}\\ +\text{ Increase in Plasma Volume}\end{array}\right)\\ \text{or}\\ \text{Gain} = \left(\text{Total Fluid Intake}\begin{array}{c}-\text{ Decrease in Plasma Volume}\\ \text{or}\\ +\text{ Increase in Plasma Volume}\end{array}\right)-\text{ Gross Water Loss}\end{matrix}\qquad(2)$$

In cases in which vomiting occurred the amount of vomitus was deducted from total fluid intake as being fluid not available for replacement of water losses Because of the difficulty of determining the amount of urine in the bladder at the beginning and end of treatment, data on urinary output are not regarded as more than approximations. For comparison of individuals, changes in gross water loss and tissue fluid were considered in terms of cc. per hour per kgm of body weight.

These equations are intended to give only approximate measurements of the changes in the water balance. Oxygen consumption was not measured and therefore the water of oxidation which might range from 200 cc. during

slower and the total air space somewhat smaller in the cabinets used in Dayton than in those used in Boston. Induction to desired fever levels was slower with the low than with the high humidities. In 7 studies conducted at Rochester N Y., fever was induced by means of radiant energy (carbon filament lamps) in cabinets with limited static air space (28)

Rectal temperature was elevated to 103 to 104° F in the patients treated with typhoid vaccine, and to 106 F or over in most of the patients treated by the physical modalities Treatment was discontinued because of collapse in Cases 4 and 17 treated by diathermy and in Cases 2, 10, and 21 treated by hot moist circulating air

TABLE I

Blood volume changes in fever produced by intravenous injection of killed typhoid organisms

Date and time	Rectal temperature	Blood volume			Change in volume		Clinical notes
		Plasma	Cell	Total blood	Plasma	Total blood	
	°F	cc	cc	cc	per cent	per cent	

CASE 11   MALE AGED 44

Date and time	Rectal temperature	Plasma	Cell	Total blood	Plasma	Total blood	Clinical notes
Apr 1 1936 4 15 p.m.	99 0	2135	1475	3160			Basal volume
Apr 2, 1936 10 07 a.m	99 2						125 million killed typhoid organisms intravenously
10 42 a.m.	99 4	2035	1440	3475	−47	−37	
11 26 a.m.	99 4	2175	1490	3665	+19	+15	Mild chill
12 54 p.m.	100 0	2135	1525	3660	0	+14	65 million killed typhoid organisms given intravenously
2 16 p.m	100.5	2095	1515	3610	−19	0	100 million killed typhoid organisms intravenously
3 16 p m	100.8	1955	1530	3610	−3.6	0	Severe chill 3 10 to 3 40 p.m
3 30 p.m.	102 0	2010	1620	3630	−12	+06	
3 53 p.m.	103.2	2140	1600	3740	+0.2	+36	
5 30 p m	102 0	2080	1450	3530	−26	−22	Repeated final volume

CASE 16   MALE AGED 31

Date and time	Rectal temperature	Plasma	Cell	Total blood	Plasma	Total blood	Clinical notes
May 27 1936 4 33 p.m.	99 0	3230	2340	5570			Basal volume
May 28, 1936 9.50 a.m	99 0						150 million killed typhoid organisms intravenously
11 37 a.m.	99 4	3185	2925	6110	−17	+97	Mild chill at 11 23 a.m
1 47 p.m	101 4	3230	2740	5970	0	+72	100 million killed typhoid organisms in travenously
3 57 p m	102 0	3140	2720	5860	−28	+52	
4·05 to 5 27 p m.							Exposed to carbon filament lamps in cabinet
4 52 p.m	103.3	3095	2455	5550	−45	−0.3	Profuse perspiration
5·28 p.m	103 4	3020	2570	5590	−6.5	+03	
6.51 p.m.	101 4	2890	2340	5230	−10.5	−5 0	Repeated final volume

CASE 9   MALE AGED 31

Date and time	Rectal temperature	Plasma	Cell	Total blood	Plasma	Total blood	Clinical notes
Jan. 19 1937 9 30 a.m.	98 8	3280	1700	4980			Basal volume
10 15 a.m.							150 million killed typhoid organisms intravenously
1 09 p.m.	100 6						150 million killed typhoid organisms intravenously
1 58 to 2 23 p.m	101 0						Severe chill
2 48 p.m.	105 4	3350	1870	5220	+21	+48	Repeated volume
5.52 p m	101.8	3240	1870	5110	−12	+26	Repeated final volume

Reactions of varying severity were encountered in a few cases as noted in Table II but treatment was uneventful in the other cases

RESULTS

The height and duration of fever, and the changes in plasma and total blood volume, expressed in terms of percentage deviation from prefebrile levels, during induction and maintenance of and recovery from fever, together with the fluid intake for each period are shown in Table II Weight changes, urinary volume, gross water loss, and tissue fluid changes are shown in Table III

Changes in plasma volume

Very little change from prefebrile plasma volume occurred in patients in whom fever was induced by typhoid vaccine, a decrease of 4 5 per cent and an increase of 0 2 per cent having been observed at the height of fever (103 2° F) in Cases 11 and 16 respectively and an increase of 2 1 per cent (at 105 4° F) in Case 9 (See Table I) During the fall in temperature, reductions of 2 6 per cent and 1 2 per cent were noted in Cases 11 and 9 respectively In Case 16, exposed to the heat of carbon filament lamps when the rectal temperature was 102 6° F a prompt and considerable decrease in plasma volume took place and continued during recovery, final plasma volume being reduced 10 5 per cent

During the induction of fever by diathermy, which required an average time of one hour and forty minutes, reductions in plasma volume were observed in all cases, ranging from 6 6 per cent (Case 12) to 19 8 per cent (Case 17) (Table II) During the maintenance of fever, plasma volume fell to lower levels than those obtaining at the end of induction in all cases, reductions ranging from 8 6 per cent (Case 12) to 32 0 per cent (Case 17) In the latter case, and in Case 4, in whom plasma volume was reduced 15 9 per cent, treatment was discontinued after temperature had been maintained at 104° F for one hour because of the development of peripheral vascular collapse At the end of recovery, plasma volume was even lower than during the maintenance period in Cases 4, 25, and 19, and slightly higher in Cases 12, 14, 17, and 23, but in no case had it returned to the prefebrile level

During the induction of fever in the "Kettering Hypertherm" which required an average time of a little over two hours, plasma volume was reduced in all cases, except in Cases 6 and 13 in whom fluids were given intravenously The most severe reductions were encountered in Cases 2, 10, and 21, amounting to 19 5, 26.9, and 16 8 per cent respectively, and treatment was discontinued in these cases shortly after induction In cases in whom fluids were given intravenously during maintenance (2, 6, 10, 13, 20 and D–3) plasma volume rose above levels obtaining at the end of induction of fever, and in cases given fluids by mouth (8, 18 24, 26 D–1, and D–2) remained at or fell slightly below the level obtaining at the end of induction During recovery to normal temperature, plasma volume tended to diminish in those cases in whom it had been raised above normal by the intravenous administration of fluids and to increase in those cases given fluids by mouth, but returned to the prefebrile level only in Cases 6, 13, and D–3 to whom fluids were given by vein and in Cases 8 and 18, given fluid by mouth

Induction of fever required an average of one hour and fifty minutes in the radiant energy cabinet and was accompanied by a reduction in plasma volume in all cases ranging from 5.9 per cent (Case R–5) to 17 0 per cent (Case R–6) Except in Case R–5 plasma volume rose above the level reached at the end of induction during maintenance of fever and in Cases R–4 and R–5 rose above the prefebrile level During the return to normal temperature, plasma volume fell, reaching levels equal to or slightly below those obtaining at the end of induction of fever except in Case R–4 in whom it was slightly higher

Changes in total blood volume

Total blood volume was increased in the three patients treated with typhoid vaccine as a result of an influx of red cells into the circulation during the febrile period in amounts large enough to offset the slight reductions that occurred in plasma volume.

In general, changes in total blood volume tended to parallel the changes in plasma volume during induction of fever in the patients treated by the physical modalities Increases in the red cells, during maintenance of fever large enough to increase total blood volume above prefebrile level in spite of a diminished plasma volume, were observed in Case 12, treated with diathermy, Case 2 treated with the "Kettering Hypertherm," and in Case R–1 treated with radiant energy After recovery to normal temperature, total blood volume was below the prefebrile level in all cases, except in Cases 6, 13, and 18 of whom the first two received all fluids by vein

Changes in water balance—gross water loss

Gross water loss averaged 510 cc., or 1 0 cc. per kgm per hour in the three cases treated with typhoid vaccine (Table III) The losses in the cases treated by the physical modalities were far greater, averaging 8 0, 9 1, and 6 4 cc. per kgm per hour in the groups treated by diathermy, hot moist circulating air, and radiant energy respectively

Within each group there were some individual variations in relation to the duration of fever and rate of fluid intake and the route by which fluids were given In none of these cases who received fluids by mouth was the rate of gross fluid loss proportional either to the number of hours of treatment, or to the rate of fluid intake Several cases treated by hot moist circulating air received fluids by vein and here again it is evident that the rate of gross water loss is not increased by prolongation of fever, nor decreased by a high rate of fluid administration (compare Cases 6, 8 and 26, Table III)

Tissue fluid loss

In cases treated by typhoid vaccine, tissue fluid changes were within the limit of experimental error, but were of considerable magnitude in the cases treated by the physical agencies All the patients treated by diathermy and radiant energy experienced losses in tissue fluid, the rate of loss being in general inversely related to the rate of fluid intake. Six of the cases treated in the "Kettering Hypertherm' experienced increases in tissue fluid, two of them having received fluid by vein and the others by mouth, but all at rates higher than received by any of the cases in the other two groups The other seven cases suffered losses in tissue fluid, the rate of loss being inversely related to the rate of fluid intake.

Blood volume changes during induction, maintenance, and recovery in artificial fever produced by diathermy in air conditioned cabinets and by radiant energy in cabinets[1]

TABLE II

Case number	Rectal temperature °F	Induction time hours	minutes	Fluid Intake Amount cc	Rate cc per hour	Rate cc per hour per kgm.	Rectal temperature above °F	Volume change Plasma per cent	Total blood per cent	Maintenance time hours	minutes	Fluid Intake Amount cc	Rate cc per hour	Rate cc per hour per kgm.	Rectal temperature °F	Volume change Plasma per cent	Total blood per cent	Recovery time hours	minutes	Fluid Intake Amount cc	Rate cc per hour	Rate cc per hour per kgm.	Volume change Plasma per cent	Total blood per cent	Total fluid intake cc per hour per kgm	Clinical notes
SEVEN CASES TREATED WITH DIATHERMY																										
4	106.0	1	10	0	0	0	104.0	-11.3	-0.7	1	00	0	0	0	104.0	-15.9	-6.5	3	00	0	0	0	-22.3	-16.1	0	Collapse
17	105.3	2	12	300	148	2.03	104.0	-19.8	-11.5	1	00	100	100	1.41	104.0	-32.0	-19.0	2	45	0	0	0	-24.7	-15.1	1.13	Collapse
14	105.0	1	35	0	0	0	105.8	-12.6	-5.1	2	00	0	0	0	105.0	-21.3	-15.7	2	00	599	240	4.23	-13.0	-7.6	1.9	Unsuccessful treatment
25	106.0	1	35	600	207	4.81	106.0	-14.4	-6.8	2	30	400	160	2.61	106.0	-17.0	-13.7	3	00	199	33	0.51	-21.6	-13.9	2.42	Vomited
19	106.0	1	50	500	270	4.14	106.0	-13.1	-11.1	3	00	500	375	5.77	106.0	-17.5	-10.0	1	45	0	0	0	-19.2	-12.0	3.07	Unsuccessful treatment
12	106.0	2	00	200	100	1.98	106.0	-6.6	+8.3	1	20	200	200	3.94	106.0	-5.0	+3.6	2	20	503	212	4.2	-7.7	-17.0	3.37	Unsuccessful treatment
23	106.0	2	00	250	250	4.62	104.0	-11.9	-6.7	3	40	575	239	4.10	99.6	-7.5	-11.5	2	20	503	215	3.97	-20.1	-7.9	4.9	First of, res less
Average		1	40	264	152	2.51				1	49	295	163	2.59				2	57	243	106	1.93			2.16	
THIRTEEN CASES TREATED WITH HOT MOIST CIRCULATING AIR IN CLOSED CABINETS																										
21	106.2	2	00	150	75	1.17	105.0	-16.9	-11.5	0	00	0	0	0	105.0	-21.2	-8.1	2	00	150	75	2.31	-20.2	-13.1	0.90	Cyanosis, weakness, muscle cramps
10	105.0	3	30	475	136	2.03	105.0	-20.9	-13.7	0	40	1250	1250	21.50	99.3	-3.2	+0.6	3	15	60	137	2.03	-12.1	-2.7	2.07	Collapse
2	106.0	2	00	340	170	2.72	105.0	-12.5	-12.9	1	00	599	516	8.27	99.6	-22.3	-14.0	3	35	0	11	0.21	-21.0	-5.0	3.39	Collapse
26	106.0	2	00	950	475	8.26	105.0	-14.7	-10.8	1	10	699	427	6.13	100.0	-21.0	-19.0	3	35	9	11	0.24	-21.0	-19.0	5.15	Delirium, struggling, profuse perspiration
24	106.0	1	50	1250	663	0.99	106.0	-13.4	-10.5	3	00	1350	427	6.13	99.9	-17.8	-9.4	3	00	570	171	2.45	-11.7	-4.8	5.67	Unsuccessful treatment
20	106.0	2	25	2000	830	10.62	106.0	-14.4	-11.7	0	40	501	501	0.89	102.0	-13.3	-11.6	3	20	409	25	3.73	-4.9	-5.2	6.73	Loss of temperature control
6	106.0	2	40	1750	657	0.32	106.0	+5.0	-8.4	1	00	1230	1230	17.73	104.2	+16.7	+6.3	3	10	1000	273	3.67	+4.2	+2.4	7.73	Unsuccessful treatment
D-3	106.0	1	20	1200	900	11.95	104.0	-12.6	-4.3	5	00	3300	660	8.73	104.0	+12.9	+4.7	1	40	499	210	3.19	-2.2	-2.5	8.16	Restless. No alarming symptoms
8	100.0	2	20	1625	697	11.20	106.0	-13.6	-8.9	1	30	1150	766	12.31	99.6	-12.3	-12.3	2	40	1235	490	7.40	-0.9	-0.4	9.70	Unsuccessful treatment
13	105.0	1	55	1800	933	15.20	105.0	+1.0	+1.1	1	00	800	800	13.05	100.1	+6.5	+3.9	2	20	600	300	4.59	-0.4	+3.5	10.1	Transient respiratory difficulty
18	104.0	2	00	1750	875	14.52	104.0	-11.8	-2.4	1	00	500	500	8.32	99.6	-11.5	-1.9	1	30	750	409	6.69	-0.5	+5.1	10.3	Unsuccessful treatment
D-2	106.0	1	35	800	503	14.21	105.0	-10.2	-6.1	5	00	2200	440	12.38	99.9	-14.2	-11.5	1	10	0	0	0	-0.4	-6.7	10.8	Unsuccessful treatment
D-1	105.1	1	40	1000	600	7.47	105.0	-11.0	-0.4	5	00	4000	800	13.05	100.7	-10.4	-8.4	1	20	700	150	2.34	-0.8	-0.9	11.0	Unsuccessful treatment
Average		2	06	1006	578	8.43				2	00	1308	653	9.63				2	35	465	101	2.77				

TABLE II—*Continued*

Case number	Induction						Maintenance						Recovery						Total fluid intake	Clinical note
	Rectal temperature	Induction time	Fluid intake		Volume change		Rectal temperature	Maintenance time	Fluid intake		Volume change		Rectal temperature	Recovery time	Fluid intake		Volume change			
	°F	hours / minutes	Amount cc	Rate cc per kilo per kgm	Plasma per cent	Total blood per cent	°F	hours / minutes	Amount cc	Rate cc per kilo per kgm	Plasma per cent	Total blood per cent	°F	hours / minutes	Amount cc	Rate cc per kilo per kgm	Plasma per cent	Total blood per cent	cc per kilo per kgm	
R-7	106.7	1 / 43	200	1.79	-14.5	-13.5	106.7	5 / 00	850	2.57	-5.7	-6.1	102.7	3 / 00	104	1.07	-19.0	-9.7	1.55	Marked, struggling, no alarming symptoms
B-3	106.7	2 / 00	300	1.90	-5.9	-4.3	106.7	6 / 00	1050	2.66	-11.8	-4.7	100.0	2 / 30	200	1.01	-11.3	-9.0	2.07	Restless. No alarming symptoms
R-4	106.7	2 / 20	400	3.36	-16.3	-14.5	106.7	13 / 00	2350	3.70	+3.4	+6.9	101.3	1 / 50	200	2.10	-10.9	-2.4	3.53	Uneventful treatment
B-2	106.7	1 / 15	500	0.93	-8.8	-8.7	106.7	13 / 00	2130	4.64	+4.68	+10.0	101.2	1 / 30	0	0	-11.9	-0.2	4.30	Uneventful treatment
R-4	106.7	1 / 25	600	3.35	-17.0	-11.8	106.7	5 / 00	1750	4.43	-10.2	-4.1	99.2	3 / 10	900	5.23	-10.1	-11.8	4.35	Weak. No alarming symptoms
R-1	106.7	2 / 15	300	4.53	-10.6	-9.0	106.7	10 / 00	2890	6.39	-4.0	+4.52	95.6	2 / 10	450	4.56	-10.5	-7.7	5.04	Uneventful treatment
Average		1 / 50	450	3.55				6 / 10	1825	4.03				2 / 00	300	2.41			3.85	

SIX CASES TREATED WITH SLIGHTLY NEGATIVE (CARBON DIOXIDE LOW) IN CABINETS WITH LIMITED WATER AIR SPACE

* Cases have been grouped according to rate of total fluid intake for each fever modality. In the interpretation of the chart attention should be paid to the method by which fluids were given (by mouth or intravenously) as well as to the amount given. Thus Cases 4, 17 and 21 had no fluid intake and the resulting dehydration has an important bearing on the clinical condition of these patients.

[1] All fluids were given by mouth except as described in footnotes
[2] Of this amount 100 cc. was 50 per cent dextrose given intravenously during this period
[3] 1350 cc. 5 per cent dextrose in normal saline given intravenously
[4] 1650 cc. normal saline slightly acidified with HCl given intravenously during induction and maintenance.
[5] 50 cc. 50 per cent dextrose given intravenously
[6] All fluids given intravenously as normal saline
[7] Cases D-1, D-2 and D-3 were treated in cabinets in which the relative humidity was maintained at about 80 per cent, the remaining cases in cabinets in which the humidity was maintained at from 30 to 50 per cent
[8] Of this amount 1500 cc was normal saline given intravenously
[9] Same patient as Case 8. All fluids given intravenously as normal saline

TABLE III

Gross water loss and changes in tissue fluid in fever induced by diathermy, in air conditioned cabinets, and by radiant energy in cabinets

Case number	Date	Age	Sex	Diagnosis	Weight	Duration of treatment		Total fluid intake		Weight change at end of treatment	Gross water loss		Fluid lost as urine	Tissue fluid changes		Clinical notes
						hours	min utes	Amount	Rate		Amount	Rate		Amount	Rate	
		years			kgm			cc	cc per hour per kgm	grams	cc	cc per hour per kgm	cc	cc.	cc per hour per kgm	

THREE CASES TREATED BY KILLED TYPHOID ORGANISMS GIVEN INTRAVENOUSLY

11	Apr 2 1936	44	M	Paresis	43.3	8	15	75	0.2	− 400	475	1.3	0	− 155	−0.4	
16	May 28 1936	31	M	Paresis	63.3	9	15	500	0.9	− 600	550	1.0	125	− 470	−0.7	Exposed to heat of carbon filament lamps
9	Jan. 10 1936	31	M	Paresis	57.5	7	45	675	1.5	+ 200	475	1.0	350	+ 160	+0.4	
	Average							416	0.9		510	1.0		− 135	−0.3	

EIGHT CASES TREATED BY DIATHERMY

4	Jan. 30 1936	39	M	Paresis	51.3	5	00	0	0	−3300	3300	12.2	0	−2770	−10.2	Collapsed
17	July 2, 1936	44	M	Paresis	71.2	5	00	400	1.1	−2510	3710	6.5	0	−1970	−5.5	Collapsed
15	May 8, 1936	37	M	Paresis	59.3	6	40	500	1.3	−2600	2300	4.1	0	−1410	−3.6	
14	Apr 22, 1936	37	M	Paresis	59.2	5	00	500	1.7	−1100	1990	6.4	0	− 970	−3.3	
25	Feb 2, 1937	45	M	Paresis	61.3	6	45	1000	2.4	−2600	3600	9.3	0	−2270	−5.5	Delirium, mania
19	July 23 1936	51	M	Paresis	65.2	5	00	1000	3.0	−2100	3100	9.5	0	−1605	−4.0	Exposed to heat of carbon filament lamps
12	Apr 0 1936	42	M	Paresis	50.5	5	40	965	3.3	−1050	2015	7.1	0	− 490	−1.7	
23	Jan. 13 1937	28	M	Paresis	54.1	7	00	1695	4.3	−1900	3595	9.4	?	−1350	−3.7	Exposed to heat of carbon filament lamps
	Average							740	2.2		2900	8.1		−1604	−4.5	

THIRTEEN CASES TREATED WITH HOT MOIST CIRCULATING AIR IN CLOSED CABINETS

21	July 8 1936	50	M	Paresis	61.2	4	00	150	0.6	−2300	2450	9.6	0	−2310	−0.1	Collapsed
16	Mar 26 1936	45	M	Paresis	66.8	7	25	1275	2.0	−1035	4775	9.7	0	−2810	−5.7	Collapsed. Recovery after 100 cc 50 per cent dextrose given intravenously
2	Dec. 13, 1935	45	M	Paresis	62.5	8	00	1600	3.4	−2800	4400	8.0	0	−2360	−5.1	Collapsed. Recovery on 1350 cc 5 per cent dextrose intravenously
26	Mar 5 1937	36	M	Paresis	57.5	5	45	1700	5.2	−2900	4600	13.0	0	−2250	−6.8	Delirium mania. 1650 cc normal saline given intravenously
24	Feb 18 1937	50	M	Paresis	69.7	8	20	3170	5.5	−1500	4670	8.1	0	−1130	−1.9	
20	July 31 1936	44	M	Paresis	78.1	4	40	2150	6.7	− 700	3150	8.1	0	− 510	−1.5	50 cc of 50 per cent dextrose intravenously
6	Feb. 20 1936	28	M	Paresis	70.5	7	20	4000	7.7	+1200	2800	5.4	510	+1310	+2.5	All fluids given intravenously
D-3	July 23 1937	20	M	Primary syphilis	75.1	8	00	4000	8.2	− 600	5500	9.2	0	− 695	−0.0	1500 cc given intravenously
8	Mar 12, 1936	43	M	Paresis	62.2	6	30	4000	9.8	+2000	2000	4.9	0	+2020	+5.0	Same patient as Case 13. All fluids given by mouth
13	Apr 16, 1936	43	M	Paresis	61.3	5	15	3250	10.1	+1300	1050	6.1	175	+1200	+4.2	Same patient as Case 8. All fluids given intravenously
18	July 9, 1936	57	M	Paresis	60.2	4	50	3000	10.3	+ 400	2600	8.0	250	+ 385	+1.3	
D-2	July 22, 1937	37	F	Paresis	50.0	8	00	5200	11.0	+ 700	4500	9.5	90	+ 460	+0.9	
D-1	July 22, 1937	32	M	Undulant fever	35.0	7	50	3000	10.8	+1250	1750	0.3	0	+1130	+4.1	
	Average							2900	7.0		3481	9.1		− 438	−1.0	

TABLE III—*Continued*

Case number	Date	Age years	Sex	Diagnosis	Weight kgm.	Duration of treatment hours	Duration of treatment minutes	Total fluid intake[1] Amount cc.	Total fluid intake[1] Rate cc. per hour per kgm.	Weight change at end of treatment grams	Gross water loss[2] Amount cc.	Gross water loss[2] Rate cc. per hour per kgm.	Fluid lost as urine cc.	Tissue fluid change[3] Amount cc.	Tissue fluid change[3] Rate cc. per hour per kgm.	Clinical notes

SEVEN CASES TREATED WITH RADIANT ENERGY (CARBON FILAMENT LAMPS) IN CABINETS WITH LIMITED STATIC AIR SPACE

Case number	Date	Age	Sex	Diagnosis	Weight	Dur. hrs	Dur. min	Amount	Rate	Wt change	Amount	Rate	Urine	Amount	Rate	Clinical notes
R 7	July 16, 1937	42	M	Paresis	63.7	9	45	1150	1.9	−2300	3450	5.7	?	−1755	−2.8	Incontinent of urine
R-5	July 7 1937	37	F	Paresis	79.0	9	30	1550	2.0	−1600	3150	4.2	?	−1160	−1.6	Incontinent of urine
R-4	June 30, 1937	42	M	Paresis	51.8	16	10	2950	3.5	−1400	4350	5.2	965	−1135	−1.4	
R-2	June 22, 1937	38	F	Tabo-paresis	38.6	14	45	2450	4.2	−1500	3950	6.8	1550	−1250	−2.2	Cord bladder Retention with incontinence
R-6	July 15, 1937	34	M	Multiple myeloma	79.4	9	25	3250	4.4	−2600	5850	7.8	300	−2080	−2.8	Received x-ray therapy at 106° F
R-3	June 24, 1937	34	M	Acute non-specific arthritis	69.6	8	12	2800	4.9	−1160	3960	6.9	675	− 650	−1.5	
R 1	Nov 29, 1936	20	F	Gonorrhea	43.8	13	40	3550	5.9	−1100	4650	7.5	110	− 915	−1.5	
Average								2530	3.9		4194	6.4		−1307	−1.8	

[1] All fluids given by mouth except as noted.
[2] Computed from Equation (1) above.
[3] Computed from Equation (2) above.

DISCUSSION

The data presented in this study cover an admittedly wide range of experimental conditions The condition of the patients cardiovascular and neurological systems varied greatly, as did their mental and emotional states In each group treated by the same physical agency, the height and duration of fever, and the amount, rate of, and mode of fluid administration purposely were varied to observe the effects of dehydration and overhydration The physical modalities of fever used each imposed a different set of environmental conditions and it is evident that individuals varied greatly in their response to comparable bouts of fever and fluid intake levels However, certain general trends were observed on the basis of which conclusions as to the physiological effects of artificially induced fever can be drawn justifiably

In the three subjects treated by typhoid vaccine the observed changes in blood volume and in the water balance were of no clinical significance It is true that the fever was of less degree and duration than in any of the patients in the other groups and this may account to some extent for the great differences in blood volume

changes, and gross water and tissue fluid losses observed between the patients treated with vaccine and by physical means However, patients in the former group sweat very little, experienced little thirst, and showed little vasodilatation. In contrast, the patients in the latter group showed pronounced vasodilatation and sweating, and it is therefore evident that the contrasting situation as regards blood volume and water balance changes is not a result of the differences in temperature levels alone

One of the major physiological effects of fever artificially induced by physical means is a diminution in circulating blood volume This diminution is owing to the uncompensated loss of large quantities of water from the blood stream, resulting in a reduction in the plasma portion of the blood and in hemoconcentration The latter effect is slightly augmented by small increases in the number of circulating red cells, never great enough to equal the loss of plasma The degree of reduction in plasma volume is determined by the difference in rate of outflow by skin, lung, and kidneys and of effective absorption of fluids administered If insufficient fluids are given, the

tissue fluids of the body are drawn upon for the maintenance of plasma volume with resultant dehydration   Blood volume may be maintained at prefebrile levels by intravenous administration of fluids, but in only 2 cases in this series was it maintained at prefebrile level when fluids were given by mouth even in large amounts   Too rapid administration of fluids by vein may result in an excessive increase in blood volume and result in cardiac embarrassment and failure

Gross water loss occurs most rapidly during the induction of fever   In all cases in this series receiving fluids by mouth, a reduction in plasma volume took place during induction, the percentage reduction from prefebrile levels being inversely related to the rate of fluid administration, and directly related to the time required for elevation of temperature   It is evident from the data presented in Figure 2 that an induction time

longer than 2 hours, by and large, involves the risk of reduction in plasma volume to critical levels

There are some differences in the degree of reduction in blood volume that can be borne by individual patients at high temperatures   It is, however, evident that *for each individual there is a definite limit beyond which further loss of fluid from the blood stream cannot be tolerated*   In those patients who experienced collapse, characterized by cyanosis, tachycardia with weak, thready pulse, marked fall in systolic blood pressure, respiratory difficulty, and even coma, total blood volume was severely diminished at the time collapse occurred   As these patients recovered, total blood volume tended to rise in proportion to the amount of fluid given during recovery   In those in whom fluids were restricted during recovery the total blood volume remained reduced but cir-
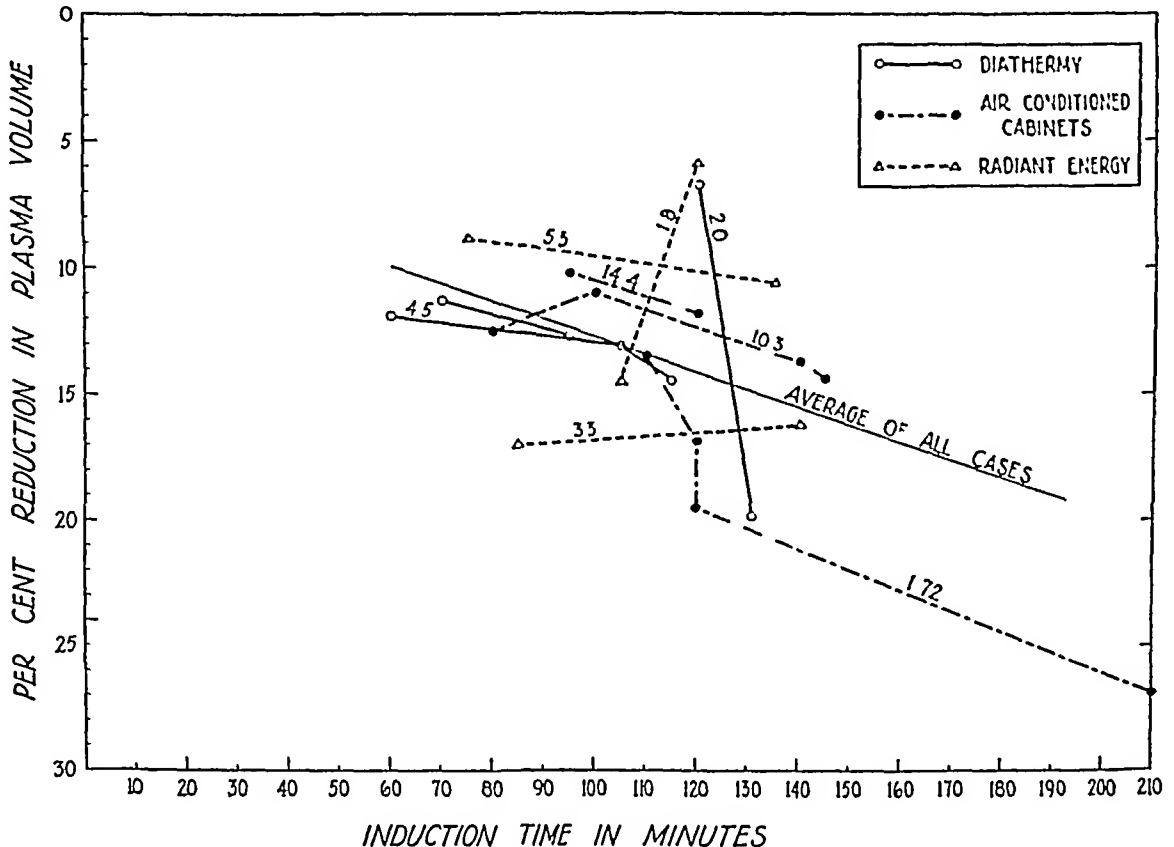

FIG 2   RELATIONSHIP OF PERCENTAGE REDUCTION IN PLASMA VOLUME TO THE TIME REQUIRED FOR FEVER INDUCTION   ALL CASES RECEIVED FLUIDS BY MOUTH

Cases have been grouped according to rate of fluid intake for each physical modality, the numerals above the connecting lines indicating the average intake in cubic centimeters per hour per kgm   The percentage reduction in plasma volume increases as the induction time is prolonged

culatory readjustment took place at the reduced level as the temperature fell to normal Thus, shock resulting from severe diminution in circulating blood volume is a potential danger in every patient treated with artificial fever Its prevention depends primarily upon adequate maintenance of the volume of the circulating blood

In Cases 8 and 13 (Tables II and III) the same patient was subjected to bouts of fever of comparable height and duration In the former instance all fluids were given by the intravenous route, and blood volume was well maintained throughout treatment. In the latter instance all fluids were given by mouth at about an equal rate, but a moderately severe reduction in plasma and total blood volume occurred during induction and maintenance of fever, even though the gain in weight and the rate of gross water loss were nearly equal in both experiments It is evident that water may pass out of the blood stream more rapidly than it can be absorbed from the intestinal tract, and this fact has an important bearing on the determination of the optimal rate of fluid administration for maintenance of blood volume

From the data presented in Table II it is evident that the fluid requirement varies with the apparatus employed Thus, the average rate of

fluid intake necessary to maintain plasma volume level and water balance is about 8, 9, and 6 cc. per hour per kgm for diathermy, the " Kettering Hypertherm," and radiant energy (28) respectively If an uncooperative or irrational patient fails to take the prescribed amount of water by mouth by the end of fever induction, it may be necessary to give fluid parenterally to maintain the water balance.

The mental state of the patient has an important relation to gross water loss and hence fluid requirement as evidenced by the diminution in blood volume and by the gross water and tissue fluid losses in Cases 26, D–3, and R–7 These patients were noisy, restless, or maniacal, took fluids less readily and tolerated treatment less well than those who were quiet and cooperative

From the data presented in Figure 3 it is evident that the prolongation of temperature at high levels does not entail a progressively increasing rate of gross water loss There is no relation between the amount of gross water loss and the amount, or rate, or route of administration of fluid as illustrated in Figure 4 Gross water loss is determined principally by the temperature and to some extent by the relative humidity of the patient's immediate environment The water loss was greatest in those cases in whom the differ-
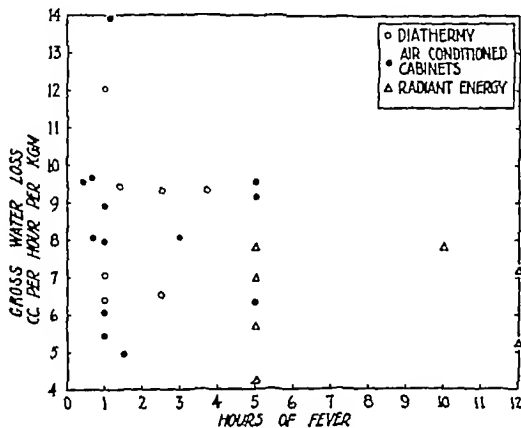

FIG. 3 RELATIONSHIP OF GROSS WATER LOSS TO HOURS OF FEVER

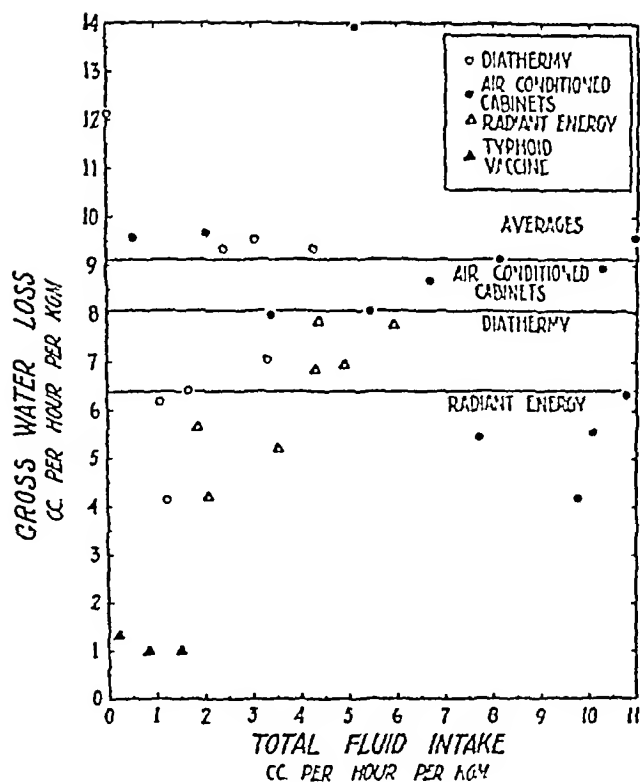The rate of fluid loss does not increase as high temperatures are prolonged.

FIG 4　RELATIONSHIP OF GROSS WATER LOSS TO TOTAL FLUID INTAKE

The rate of gross water loss is not decreased by the administration of large amounts of fluid, by mouth or by vein

ential temperature between the patient's body and his environment was highest, and least in those cases in whom it was lowest No conclusive evidence was obtained in this study that relative humidity alone plays a decisive role In patients treated in the "Kettering Hypertherm" the rate of gross water loss was the same, by and large, in those instances where the relative humidity was kept at 80 per cent as in those where it was only 40 per cent, and the relation of tissue fluid loss to rate of intake was the same (Cases D–1, D–2, and 18, and D–3, 8, and 13, Table III) No measurements of relative humidity were obtained in the radiant energy cabinets in which the air was static, but it is doubtful if it was over 80 per cent at any time, and yet the rate of water loss was less than in the cases treated in the "Kettering Hypertherm" It seems significant that the dry bulb temperature of the air in the radiant energy cabinet could be dropped to nearly the patient's own temperature, whereas it had to

be maintained around 120° F in the "Kettering Hypertherm" to obtain high humidity of the circulating air In a moving air stream at a temperature of 120° F or above, even at a relative humidity of 80 per cent, evaporation can still occur

The rate of loss or gain in tissue fluids is directly related to the rate of fluid intake as illustrated in Figure 5 It is striking that in all those cases, in which untoward effects were encountered, the loss of tissue fluid exceeded 5 cc per hour per kgm of body weight *This figure may be said to represent the limit of tolerance to loss of tissue fluid at high body temperatures*

Finally, it should be said that the assumption by many workers that the blood volume remains constant during artificial fever goes far toward invalidating their interpretations of changes in blood constituents It must be evident that, if changes in the total blood volume of the magnitude we have shown to occur are not taken into account, simple determinations of the concentration of any blood constituent are meaningless as a basis for any understanding of the physiology of artificial fever

CONCLUSIONS

1 Artificial fever produced by physical means is characterized by a large gross water loss from the blood stream by way of the skin and lungs

2 The rate of water loss may be far more rapid than the rate of absorption from the intestinal tract or tissue spaces, resulting in varying degrees of reduction in plasma and total blood volume

3 Water is lost most rapidly during the induction of fever during which the degree of reduction in blood volume is inversely related to the amount of fluid given, and directly related to the time required for elevation of temperature

4 While individuals vary in their response to artificially induced high temperature, there exists for each individual a blood volume level beyond which further reduction in volume leads to peripheral vascular collapse

5 The rate of gross water loss during maintenance is not directly related to the duration of fever or to the amount of fluid or the method by which it is administered, but is related to the difference in temperature between the patient's
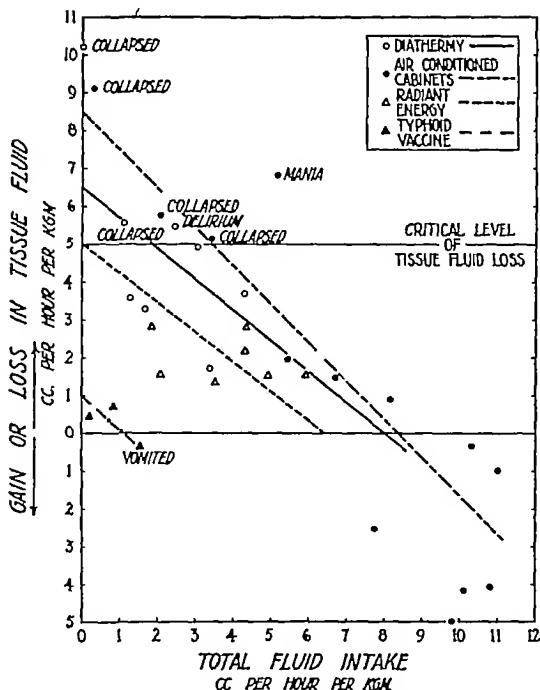
The amount of fluid withdrawn from or entering the tissue
spaces is directly related to the rate at which fluids are given.
A net loss of tissue fluid at a rate exceeding 5 cc. per hour per
kgm. of body weight cannot be tolerated at high temperatures

body and the environmental air and to the relative
humidity thereof

6 The rate of loss of tissue fluids is directly
related to the rate of fluid intake, and tissue fluid
loss at a rate exceeding 5 cc. per hour per kgm
involves the risk of serious collapse

7 The prevention of shock in artificial fever
therapy is dependent upon the giving of fluids in
amounts and by routes adequate for the main-
tenance of the blood volume and water balance

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EFFECT OF INSULIN ON THE CONCENTRATION OF URIC ACID IN THE BLOOD

By EDWARD F ROSENBERG [1]

(From the Mayo Foundation Rochester Minn)

In the past, opportunity to study the hypoglycemic state in human beings has been difficult to obtain   Before the introduction of the insulin hypoglycemia treatment of schizophrenia, the hypoglycemic state was considered to be dangerous and only a few observations were made by deliberately inducing this state in man.   In January, 1937, the treatment of schizophrenia by the insulin hypoglycemia method was undertaken at the Rochester State Hospital   The patients were normal, physically, but had a functional mental disease, they thus provided an excellent chance to observe the effects of the injection of insulin and insulin hypoglycemia   The following study on the relation between the concentration of uric acid in the blood and the administration of insulin is a portion of the work done on the chemical changes accompanying hypoglycemia among these patients

If one administers a large dose of insulin and allows hypoglycemia to develop, the concentration of uric acid in the blood drops markedly   The extent of the drop varies, but it may reach large proportions (Table I)

[1] Fellow in Medicine.

Insulin hypoglycemia decreases the concentration of uric acid in the whole blood, serum, and plasma   The decreases differ, however, in the different components of the blood (Table II)

Table III shows the concentration of uric acid and sugar when insulin was not administered   These control studies, which do not show any significant spontaneous alteration in the concentration of uric acid in the blood, would seem to confirm the impression that there is a causal relationship between the administration of a large dose of insulin and lowering of the value for the uric acid in the blood

These studies led to an inquiry into the question as to whether the ability of insulin to cause a drop in the value for the blood sugar, or some other property of insulin, is responsible for the change. To study this question a large dose of insulin was given to fasting patients   This dose was equal to or greater than the amount necessary to produce coma. Following this, sufficient carbohydrate to prevent the appearance of hypoglycemia was administered   The lowering effect of insulin on the blood sugar was counteracted by the giving of approximately 25 grams of chocolate

TABLE I

Effect of injection of insulin on the concentration of sugar and uric acid in the blood in three cases of schizophrenia

Date of analysis	Case	Units of insulin administered at 7 a.m.	Sugar*			Uric acid†			Maximal decrease in concentration of uric acid
			7 a.m	9 a.m.	11 a.m	7 a.m	9 a.m	11 a.m	
1937			mgm per 100 cc of blood	mgm per 100 cc of blood	mgm per 100 cc of blood	mgm per 100 cc. of blood	mgm per 100 cc. of blood	mgm per 100 cc of blood	per cent
Mar 23	1	65	117 9	56 9	44.5	3.5	2 0	1 1	68.5
Mar 24	2	100	100 0	55.5	25 0	4 4	4 0	3 6	18.2
Mar 24	1	80	125 0	60 6	27.3	2 7	2.2	1 9	29 6
Mar 29	2	80	117 0	51 4	29 2	4 8	4 0	3 1	35 5
Mar 30	2	100	106.3	57 1	25 1	3 9	3 4	2 9	25 6
Mar 30	3	90	96 6	53.3	24 7	3 4	3 0	2.5	26.5
Apr 1	1	80	103 1	30 3	26.2	3 1	3 0	2 9	6 4
Apr 1	2	100	90 9	28 9	21 7	3.5	2.9	2.8	20 0
Apr 1	3	80	89 7	29 4	23 4	3 1	2 7	2 6	16 1

* Determined by method of Folin (16)
† Determined by method of Folin (17)

TABLE II

Effect of administration of 100 units of insulin * on the concentration of uric acid and sugar in the blood in Case 2

		Concentration			Maximal decrease in concentration of uric acid
		7 a.m	9 a.m	11 a.m	
		mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	per cent
Uric acid	In whole blood	2 0	1 4	1 3	35 0
	In plasma	4 0	3 0	2 3	42 5
	In serum	5 4	2 2	1 0	81 4
Sugar in whole blood		106 3	35 7	30 4	

* 100 units of insulin administered at 7 a m , April 2, 1937

TABLE III

Concentration of uric acid and sugar in the blood when insulin was not administered

Date of analysis	Case	Sugar			Uric acid			Maximal change in concentration of uric acid	
		7 a.m.	9 a.m.	11 a.m.	7 a.m.	9 a.m.	11 a.m.	Increase	Decrease
		mgm per 100 cc. of blood	mgm. per 100 cc. of blood	mgm per 100 cc. of blood	mgm. per 100 cc of blood	mgm. per 100 cc of blood	mgm per 100 cc of blood	per cent	per cent
1937									
Mar 25	1	105.3	100 4	95.2	2.5	2 4	2.9	16	
Ma. 25	2	100.5	95.2	94 6	3 8	3 6	3.2		15 8
Mar 25	3	102.6	98.5	97 6	2.6	2 7	2.3		11.5

candy each 15 to 30 minutes after the study was begun (Table IV) Clinical evidence of hypoglycemia did not appear in any of these patients during the studies , nevertheless, the value for the uric acid in the blood fell markedly It seems,

therefore, that one can administer a large dose of insulin, prevent the development of hypoglycemia by the administration of carbohydrate, and yet produce a considerable drop in the concentration of uric acid in the blood

COMMENT

The literature includes the following reports of studies on the interrelationship of carbohydrate and purine metabolism In 1923, Remond and Rouzaud (1) observed a relationship between the concentration of dextrose and uric acid in the blood They stated that the concentration of uric acid is decreased by administration of carbohydrate In 1924 Lennox (2) noted that the concentration of uric acid in the blood is increased by starvation and diminished again when the feeding of carbohydrate and protein is resumed Lockie and Hubbard (3) found that diets high in fat and low in carbohydrate induce an increase in the concentration of uric acid in the blood of patients who have gout and that diets high in carbohydrate lower the concentration in these cases Quick (4) has shown that ketosis or a lack of antiketogenic substances can cause a retention of uric acid in the body

Tashiro (5) induced a decrease in concentration of uric acid in the blood of geese by the injection of insulin whereas Liotta (6) reported an increase in the dog Kurti and Gyorgyi (7) observed that the time necessary to excrete a given amount of uric acid is prolonged by administration of insulin but Chrometzka (8) stated that he had been unable to influence purine metabolism with insulin Taubmann (9) described an increase in

TABLE IV

Concentration of uric acid and sugar in the blood in cases in which sugar was given at intervals of 15 to 30 minutes after the administration of insulin

Date of analysis	Case	Units of insulin administered	Sugar			Uric acid			Maximal decrease in concentration of uric acid
			7 a.m	9 a.m	11 a.m	7 a.m	9 a.m	11 a.m	
			mgm per 100 cc of blood	mgm per 100 cc of blood	mgm per 100 cc of blood	mgm per 100 cc of blood	mgm per 100 cc of blood	mgm per 100 cc of blood	per cent
1937									
May 2	4	95	125 0	81 6	74 1	2 3	2 0	1 1	52 0
May 3	4	95	105 3	67 1	71 4	3 5	2 7	1 7	51 5
May 4	5	100	111 1	62 5	69 0	2.2	1 5	1 8	18 2
May 4	6	65	90 9	83.3	66 6	1.3	1.3	1 1	15.3
May 4	7	70	76 9	63 9	60 6	2 4	1.5	1 2	49 0
May 4	8	100	125 0	76 9	71.2	2.5	2 2	1 7	32 0
May 4	9	90	86 9	60 6	64.5	2 9	1 9	1 5	48 2

excretion of allantoin in dogs following injection of insulin whereas Ogawa (10) reported a decrease. Buadze (11) stated that in dogs he had induced an increased excretion of uric acid accompanied by a decreased output of allantoin by injection of insulin

A series of studies (12, 13 14, 15) which have appeared from Chaikoff s laboratories bear on the subject of the relation of purine and carbohydrate metabolism in dogs These workers have shown that either injection of insulin or epinephrine increases excretion of allantoin by ordinary breeds of dogs and increases excretion of uric acid in Dalmatian dogs They found that insulin could not induce these changes in the absence of the adrenal glands, or if the effect of the insulin was counteracted by carbohydrate feeding

A method of bringing about a marked fall in the concentration of uric acid in the blood, such as has been observed following the administration of insulin, in the present investigation, may have some therapeutic usefulness in cases of gout Experiments to determine this are under consideration

CONCLUSIONS

1 Subcutaneous injection of approximately one to two units of insulin per kilogram of body weight causes a marked fall in the concentration of uric acid in the blood of man

2 The drop in concentration of uric acid is independent of the appearance of hypoglycemia. It occurs when hypoglycemia is permitted to develop and also when the lowering effect of insulin on the concentration of blood sugar is counteracted by administration of carbohydrate

This work was carried on under the direction of Dr B F Smith, Superintendent of the Rochester State Hospital, and Drs F P Moersch and R M Wilder of The Mayo Clinic.

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STUDIES OF THE CIRCULATION IN PATIENTS SUFFERING FROM SPONTANEOUS MYXEDEMA[1]

By HAROLD J STEWART, JOHN E. DEITRICK AND NORMAN F CRANE

(From the New York Hospital and the Department of Medicine Cornell University Medical College New York City)

(Received for publication December 22, 1937)

It has been demonstrated that changes occur in the heart in the presence of myxedema In 1918, Zondek (1) first described the large sluggish heart, bradycardia, and certain alterations in the electrocardiogram Since then Assmann (2) has shown that the use of thyroid extract results in the reduction in the size of the heart, but that digitalis is without effect in this respect Hallock (3) reported fall in blood pressure, decrease in the size of the heart, weight loss, increase in vital capacity, and alterations in the electrocardiogram following the administration of thyroid extract Means (4) studied the relationship between the basal metabolic rate the pulse, and the cardiac output. Venous pressure is not significantly affected by the use of thyroid extract (5) Altschule and Volk (6) studied the volume output, the work of the heart, and the circulation rate in hypothyroidism induced by total ablation of the thyroid gland They found the minute volume decreased in seven patients, the arteriovenous oxygen difference of the blood increased, and the blood velocity in most instances slower than normal

We have had occasion to make studies of four patients suffering from spontaneous myxedema Although previous workers have studied individual functions of the heart and circulation in this disease, observations have not been recorded in which the several functions have been correlated in the same patient both before and during treatment with thyroid extract[2]

METHODS

All patients were free of the signs of congestive heart failure. Observations were made in the morning while the patients were in a basal metabolic state. Measure ments of the cardiac output were made by the acetylene method, three samples of gas being taken as recommended by Grollman (7), and further elaborated by Grollman, Friedman Clark, and Harrison (8) During this measurement the patients were sitting in a steamer chair (angle 135 degrees) with legs extended. They were trained to carry out the procedures beforehand. While resting quietly the radial pulse was counted at intervals of five minutes. At the end of one-half hour the acetylene air mixture was rebreathed Three samples of the gas were then taken during each rebreathing period for estimation of the arteriovenous oxygen difference. Three periods of rebreathing were carried out on each patient. Shortly afterward, the oxygen consumption was measured with a Benedict Roth spirometer After a short pause, the vital capacity was measured and the height and weight recorded Then the patient rested again, lying down. In succession, sufficient time being allowed between each procedure for the patient to return to a basal state, an electrocardiogram was taken, the arm to tongue circulation time recorded, the venous pressure estimated, and the blood pressure measured. Finally an x ray photograph of the heart was made at a distance of two meters

The arm to tongue circulation time was estimated by the use of decholin 5 cc. of a 20 per cent solution were injected rapidly (1 to 2 seconds) through an 18 gauge needle into an antecubital vein while the patient was lying quietly in the prone position. This was repeated in one and one half minutes after the response to the first test had been elicited. The time was recorded from the beginning of the injections until the patient perceived the bitter taste The injection time was also recorded since, however, the response may come with a minimum amount of the drug the time which we have used was taken from the start rather than from the conclusion of the injection. The same vein either right or left was used in each patient for the injections.

The venous pressure was measured by the direct method (9) using a large antecubital vein the vein being placed on a level with the right auricle. The apparatus consisted of an L-shaped tube of glass attached to a three way stopcock, syringe and an 18 gauge needle. The apparatus was filled with a solution of sterile, normal saline, a venipuncture performed, and the direct pressure readings recorded. Normal pressures with this apparatus range from 4 0 to 9 0 cm. of saline. The same vein was used for subsequent observations and the other one for the estimation of the circulation time.

X ray photographs of the heart were   the

[1] Read by title before the Twenty Eighth Annual Meeting of the American Society for Clinical Investigation Held in Atlantic City, New Jersey May 4 1936

[2] We wish to express our indebtedness to the Metabolism Division of the Department of Medicine for its cooperation and permission to study these patients.

TABLE I

Data of five patients suffering from myxedema

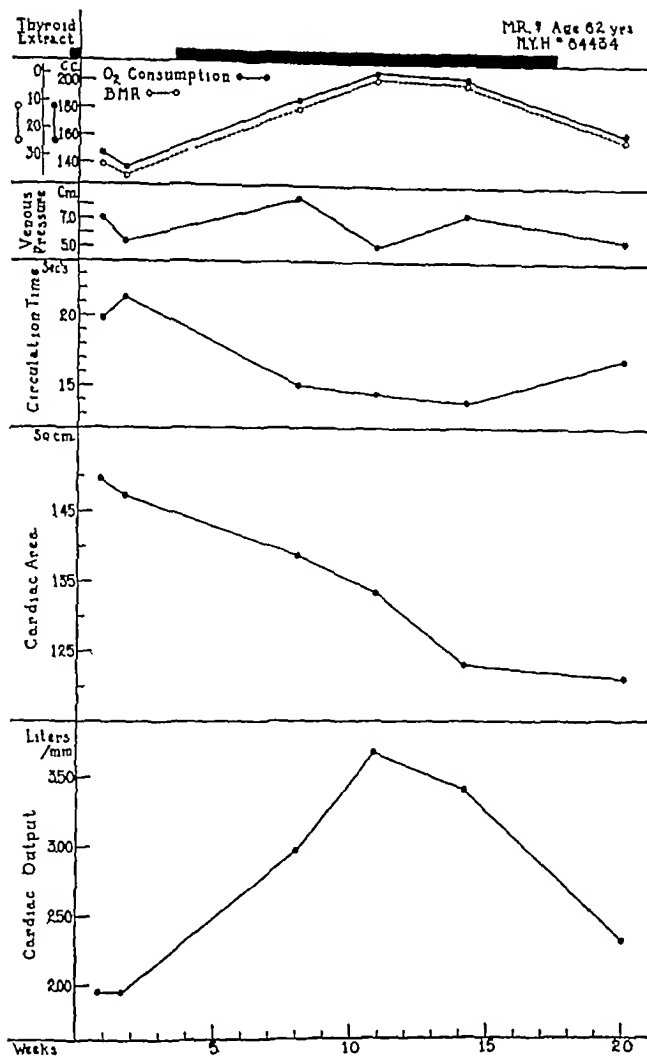| Case number name age and sex	Date	Pulse rate	Oxygen consumption	Basal metabolic rate	Arterio venous oxygen difference	Cardiac output	Cardiac output pulse	Heart rate	Cardiac output	Cardiac area	Cardiac volume	Arterial pressure	Left ventricular work	Circulation time	Venous pressure	Vital capacity	Red blood count	Hemoglobin
		per min	cc. per min.	per cent	cc.	liters per minute	liters per m. per minute	per minute	cc. per beat	sq cm	cc.	mm. Hg	gram meters per beat	seconds	cm. saline	cc.	millions	per cent
Case 1 M R, Number 54151, 45 yrs	Feb 7 1935	152	147	−33	73.6	1.95	1.03	74	26.3	149.6	1657	120/80	36.0	19.8	7.0	1900	4.7	100
	Feb 12 1935	152	137	−37	70.3	1.96	1.03	68	28.7	147.2	1628	118/80	39.0	21.3	6.3	2100		
	Feb 24 1935	Thyroid extract started																
	Mar 9 1935	152	157	−12	62.9	2.97	1.63	78	39.0	135.7	1490	124/78	53.0	15.0	8.5	2000		
	April 17 1935	153	20?	−1	56.2	3.68	2.04	76	48.0	133.3	1403	120/52	56.0	14.4	5.0	2100		
	May 10 1935	175	203	−3	50.5	3.41	1.92	80	42.6	123.1	1261	140/64	59.0	13.8	7.3	2100		
	June 2 1935	Thyroid extract discontinued																
	June 27 1935	179	16?	−22	70.2	2.31	1.29	70	33.0	121.1	1217	108/58	37.0	16.0	5.4	2300		
Case 2 J P, Number 54153, 49 yrs	Mar 1 1935	165	172	−20	73.5	2.20	1.31	64	34.0	144.9	1606	131/90	52.0	19.6	7.3	2800	4.1	100
	Mar 7 1935	Thyroid extract started																
	April 6 1935	167	189	−5	65.5	2.89	1.73	70	41.0	146.3	1612	160/90	70.0	18.3	9.5	2700		
	Jun 21 1935	162	207	+7	58.9	3.51	2.16	64	55.0	131.7	1378	122/76	74.0	11.5	8.8	2500	4.4	85
Case 3 M M, Number 54154, 9 yrs	May 23 1935	165	120	−39	82.0	1.46	0.87	66	22.0	113.7	1105	130/80	31.0	22.6	6.8	1900	3.8	73
	Jun 1 1935	Thyroid extract started																
	Jun 27 1935	165	153	−21	68.0	2.25	1.36	75	30.0	104.5	973	122/70	39.0	19.0	6.8	2300		
	July 12 1935	163	170	−12	60.3	2.83	1.73	90	31.4	102.0	940	112/68	38.0	17.6	5.9	2250		
Case 4 A H, Number 11347, 2 yrs	Feb 3 1935	191	166	−32	67.8	2.45	1.26	68	36.0	151.9	1757	105/78	45.0	14.6	9.9	3200	3.7	67
	Feb 9 1935	Thyroid extract started																
	Mar 4 1935	190	180	−26	66.7	2.70	1.42	84	32.0	149.4	1670	112/80	42.0	12.6	8.7	3200	4.3	74
	Mar 18 1935	185	209	−10	65.4	3.20	1.72	82	40.0	117.9	1167	116/74	52.0	11.2	7.6	3200	4.3	74
	April 5 1935	181	225	0	61.0	3.71	2.05	92	43.0	115.6	1132	128/80	61.0	9.9	7.7	3100	4.4	74
Case 5 M S, Number 11341, 4 yrs	April 18 1935	Thyroid extract started																
	April 24 1935	161	172	−20	73.5	2.31	1.43	80	29.0	137.3	1468	156/108	52.0			2950	4.9	100
	Oct 10 1935	On thyroid extract																
	Oct 19 1935	163	175	−16	74.0	2.41	1.48	68	35.0	134.8	1448	158/98	59.0	20.2	5.8	3200		

FIG. 1 THE EFFECT OF THYROID EXTRACT ON THE OXYGEN CONSUMPTION, THE BASAL METABOLIC RATE, THE VENOUS PRESSURE, THE CIRCULATION TIME, THE CARDIAC AREA, AND THE CARDIAC OUTPUT OVER A PERIOD OF 20 WEEKS IN M R., CASE 1

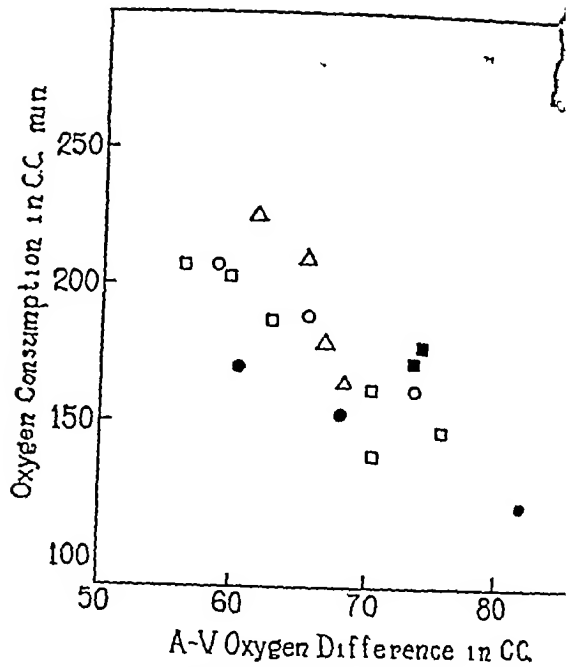The last measurements were made 18 days after thyroid extract had been discontinued.

FIG 3 CORRELATION BETWEEN THE OXYGEN CONSUM
TION AND THE ARTERIOVENOUS OXYGEN DIFFERENCE

As the oxygen consumption of each individual increas
in thyroid therapy the arteriovenous oxygen differen
becomes less

ncreases   In a similar fashion, there is a linea
correlation between the basal metabolic rate and
he arteriovenous oxygen difference (Figure 4),
ince the arteriovenous oxygen difference becomes
ess with the rise in the basal metabolism until
ormal relationships are established at 60 0 cc.
rteriovenous oxygen difference, and zero basal
metabolic rate   Finally, a linear relationship L
ound in these patients between the circulation
me and the cardiac output per minute, as the
cardiac output increases, the circulation time be
omes shorter (Figure 5)

The venous pressure in these patients was not
elevated during the myxedematous state and
owed no uniform changes as the basal metabolte rose to normal

The size of the heart decreased markedly on
yroid therapy in all patients except in Case ?,
attempt was made to raise the metabolic r:
this patient to normal by use of thyroid ex
ct   The greatest diminution in size occurred
Case 4 amounting to 25 per cent of the init
e measured before thyroid extract was a
nistered   The smallest reduction occurred
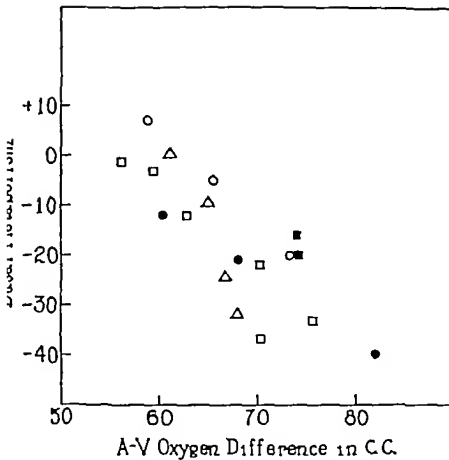se 2 amounting to 9 7 per cent of the in

A-V Oxygen Difference in C.C.

FIG 4 CORRELATION BETWEEN BASAL METABOLIC RATE AND THE ARTERIOVENOUS OXYGEN DIFFERENCE

As the basal metabolic rate increases the arteriovenous oxygen difference decreases

ize The case of M R (Case 1) serves to illustrate these changes (Figure 6)

DISCUSSION

The myxedematous state in these patients was associated with a low basal metabolic rate with decrease in cardiac output per minute and per beat with increase in the arteriovenous oxygen difference and slowing of the velocity of the blood flow This was a reversible state since the administration of thyroid extract was associated with alterations toward the normal levels of these functions and with shrinking in the cardiac size

The finding of a widened arteriovenous oxygen difference during the myxedematous state is somewhat puzzling In the myxedematous state it appears that, even though the circulatory needs of the body are markedly lowered the heart does not maintain a circulation adequate for these lowered requirements since the arteriovenous oxygen difference increases In short the velocity of the blood flow may be so slow that greater amounts of oxygen are removed from each unit of blood than normally occurs Boothby and Rynearson (13) proposed the hypothesis that in the hyperthyroid state there is present in the or-

ganism a special circulatory stimulant, which causes a greater increase in the circulation rate than occurs in a normal subject as the result of a corresponding increase in oxygen consumption due to work In the myxedematous state it may be possible that the opposite is the case, namely that the circulation becomes abnormally slow in comparison to the oxygen consumption because of the lack of such a substance. Thyroid therapy in this state however apparently supplies a substance which in addition to increasing the oxygen consumption of the body also stimulates the circulatory apparatus to return to such an efficient state that the arteriovenous oxygen difference becomes normal Our observations lend weight to Boothby and Rynearson's hypothesis The slowing of the velocity of the blood flow and the decrease in cardiac output in the myxedematous state are apparently brought about by factors different from those present in congestive failure (14)

In Case 4 A H, there occurs an additional factor which leads to interesting observations This patient, as has been stated before was anemic. Although the basal metabolic rate was low (— 39 per cent) in the presence of a rather severe myxedematous state, the arteriovenous
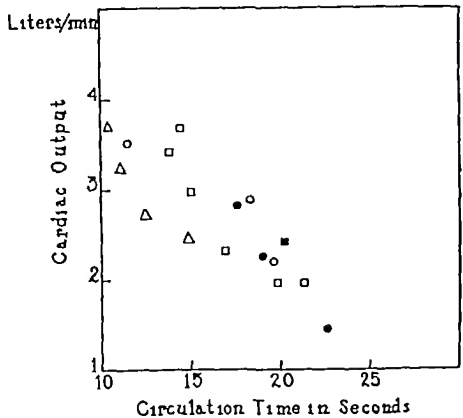

FIG 5 LINEAR RELATIONSHIP BETWEEN THE CARDIAC OUTPUT AND THE CIRCULATION TIME AS IMPROVEMENT OCCURRED WITH THE USE OF THYROID EXTRACT

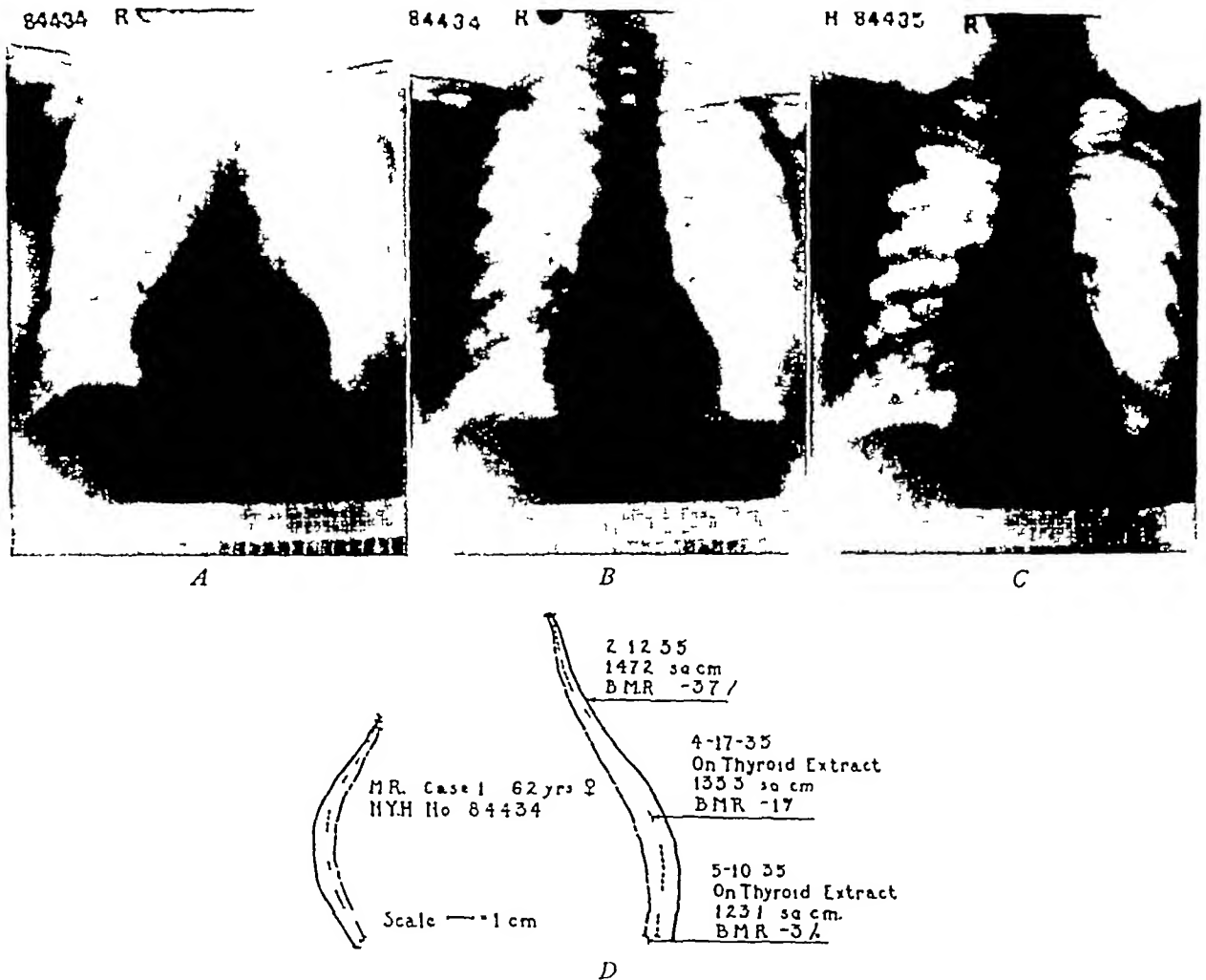As the cardiac output increases the circulation time becomes shorter

FIG 6   PHOTOGRAPHS OF X-RAYS OF M R, CASE 1

Photograph A was taken on February 12 1935, at a time when the basal metabolic rate was — 37 per cent, before thyroid extract was given   Photograph B was taken on March 7, 1935, after the basal metabolic rate had risen to — 17 per cent on the administration of thyroid extract   Photograph C recorded the size of the heart on May 10 1935 when the basal metabolic rate was 0 per cent   D represents outlines of the heart traced from the x ray photographs A B and C on thin paper and superimposed in the manner shown

oxygen difference was at the upper limits of normal and moreover the velocity of blood flow was well within normal limits   It is recalled that in the other patients the arteriovenous oxygen difference was increased as well as the circulation time   It has been shown by Stewart Crane and Deitrick (15) and others (16, 17) that anemia speeds up the circulation rate   On the basis of this cvide ce it appears that this factor was accountable for the normal arteriovenous oxygen difference in this patient in whom we would have otherwise expected an increase   If this is the case it means that the circulatory appa-

ratus in myxedema is able to respond in a normal fashion to factors other than thyroid extract   The data on this patient lend evidence to our belief that the wide arteriovenous oxygen difference in myxedema is owing to the slow velocity of blood flow

Another question which might be raised is whether the entire circulatory system is affected by thyroid therapy or whether the heart is especially susceptible   It appears from our correlations that the decrease in the circulation time and the decrease in the arteriovenous oxygen difference following therapy correspond very

closely to the decrease in heart size and to its increased output and work per beat (see p 247)

We had the opportunity of studying one of the patients, M.R , Case 1, after thyroid extract had been discontinued, and to make deductions relating to the duration of its effect June 20, 18 days after thyroid extract had been discontinued (Figure 1), the heart remained small, although the basal metabolic rate was now—22 per cent, nevertheless it was less efficient than when it had been larger in size with the basal metabolic rate —1 per cent or —3 per cent This indicates that heart size alone, in this state, is not the only factor regulating its efficiency It is apparent, by inference, that thyroid extract might have affected the muscle directly when it was given or that in its absence the circulation had lacked the stimulating factor In spite of this decrease in efficiency, the circulation was nevertheless still considerably more adequate than it was before thyroid therapy was first instituted It appears that the effect of thyroid therapy persists, at least longer than 18 days (Figure 7) since the work of the heart in proportion to its size did not drop along the same line as it had risen on thyroid therapy These differences are perplexing and we believe warrant further study In short, certain effects of the lack of thyroid hormone probably appear only after a long period of deprivation while the changes in basal metabolic rate take place in a shorter time.

Electrocardiograms of these patients were taken at frequent intervals both before and after the institution of therapy In the patients suffering from spontaneous myxedema, before therapy was begun, the QRS complexes were of low amplitude and slightly split The T-waves in all three leads were of low amplitude. T_1 was negative in one (Case 1) and diphasic in another patient (Case 2) and ' coved " in both T_2 was diphasic in three patients (Case 1, Case 2 and Case 3) There was right axis deviation in two (Case 3 and Case 4) and left axis deviation in two (Case 1 and Case 2) The P–R conduction time was prolonged in one (Case 4) and at the upper limits of normal in two others (Case 2 and Case 3) The chest lead [a] was characterized

[a] The chest lead was derived from the right arm electrode placed just within the apex and the left arm electrode placed in the interscapular region (18)
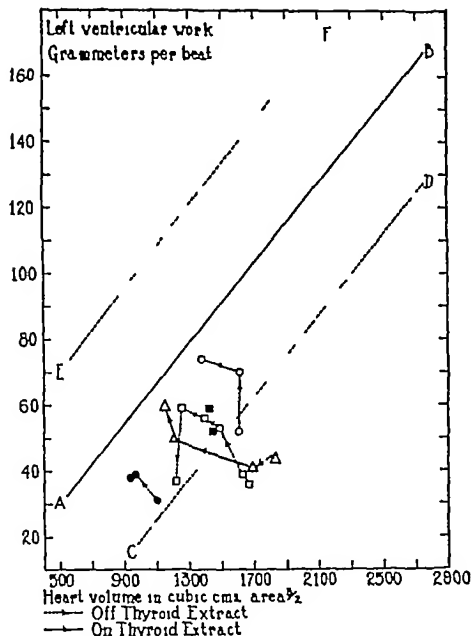

FIG 7 LEFT VENTRICULAR WORK PER BEAT AND CARDIAC VOLUME

The data from Table I relating to work of the left ventricle per beat are plotted against the corresponding cardiac volumes. Line AB represents the best line, the regression of the work on the area, defined by Starr, Collins and Wood (12 Figure 2) on the basis of a statistical treatment of data from a control group of cases. Lines CD and EF are placed by these authors at a distance of twice the standard deviation from AB It appears from their observations that a patient falling within the zone $CD-EF$ has a normal circulatory function that is to say the work of the heart is commensurate with its size on the other hand, they found that the values relating to patients who had suffered from cardiac decompensation, fell in a zone below CD In the myxedematous state before thyroid extract was administered, three of the patients studied fell outside the line CD while the other two fell just within the line CD After therapy all the patients moved up into the normal zone, toward the line AB

by very small Q waves, moderately low R-waves, and low voltage T-waves which were negative, but with a slight positive phase in two instances (Case 1 and Case 3)

A.H.
119047 N.Y.H.

LEAD I   LEAD II   LEAD III

Jan. 30,1936
Before Thyroid
Extract

March 5,1936
On Thyroid
Extract

April 8,1936
On Thyroid
Extract
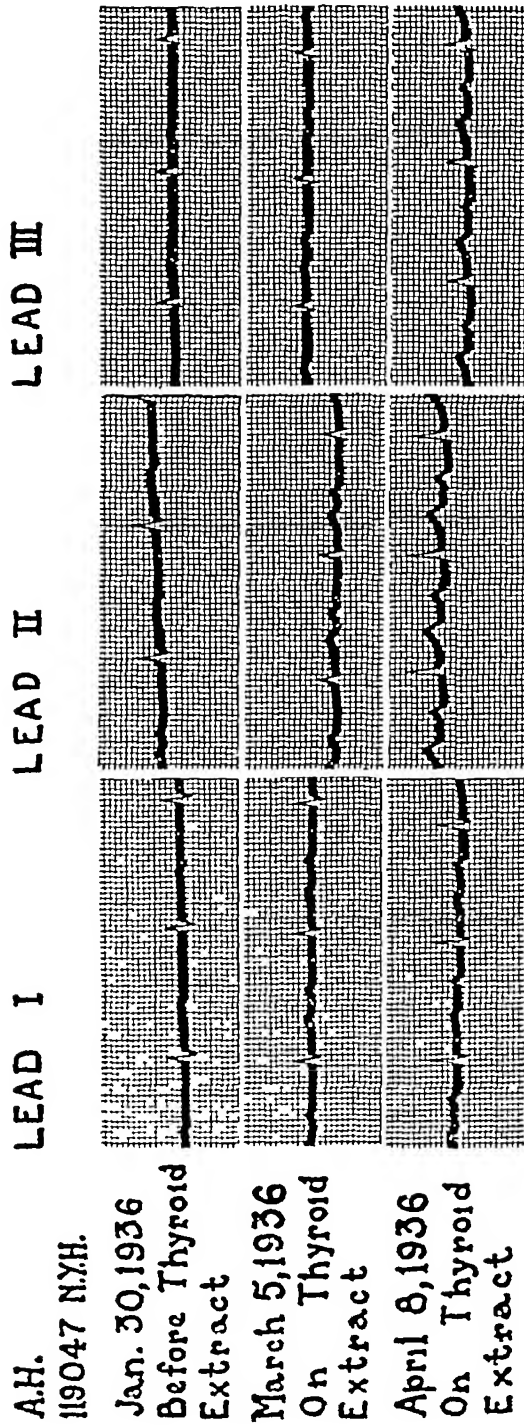
FIG 8 CHANGES OCCURRING IN THE ELECTROCARDIOGRAM OF A H, CASE 4, WHEN GIVEN THYROID EXTRACT

The first record was taken on January 30, 1936, before thyroid extract had been administered when the basal metabolic rate was −36 per cent The second record was taken on March 5, 1936, after the extract had been given for 24 days and the basal metabolic rate had increased to −24 per cent The third record was taken on April 8, 1936, 33 days later still when the basal metabolic rate had risen to 0 per cent Divisions of the abscissae equal 0 04 of a second The standardization is such that 1 cm deflection of the string is equivalent to 1 millivolt The original curves are sharply contrasted black and white, no half tones are lost by this method of reproduction The electrocardiograms are reduced to two thirds their natural size

Following the administration of thyroid extract, the QRS complexes and the T-waves increased in amplitude. The two patients exhibiting right axis deviation beforehand, now showed left axis deviation The P–R conduction time decreased in all four cases, even though it was not prolonged during the myxedematous state In the chest lead the Q-waves increased in amplitude and the T-waves became more negative in two cases (Case 1 and Case 3) The electrocardiographic records of A H (Case 4) serve to illustrate certain of these changes (Figure 8)

In the case of M. R. (Case 1) there was occasion to observe the electrocardiograms first before thyroid extract was given, then during the administration of this extract, when she exhibited the alterations which have just been described Now when the extract was no longer given, the configuration of the electrocardiogram reverted to its earlier type, characterized by low QRS complexes and flat low T-waves Once again when the extract was given, changes were recorded in the electrocardiograms as before.

In the case of M S (Case 5) who had experienced total thyroidectomy, the use of thyroid extract was associated with increase in amplitude of the T-waves and in changes in the chest lead similar to those which we have described

The electrocardiographic characteristics of myxedema appear to be low amplitude of the QRS complexes and of the T-waves in the three standard leads, as well as in the chest lead Moreover, the Q waves in the chest lead are small The administration of thyroid extract results in increase in amplitude of the QRS complexes and of the T-waves

The nature of the cardiac enlargement [4] in the myxedematous state is not known Whether there is dilatation of the chambers of the heart associated with altered venous return (although our observations show no significant alteration) or increase in circulating blood volume, or whether enlargement is a consequence of alterations in the muscle fibers and tissue spaces are matters which have not been settled Most authors agree that such a heart is microscopically very little different from normal heart muscle, although all call attention to the large, flabby organ when the gross specimen is examined

That it is not hypertrophy of the organ is apparent since its size decreases so readily when thyroid extract is given, in short, it is a reversible reaction Moreover, the heart in this state is a sluggish organ and accomplishes less work than normal at each beat (Table I) We have calculated the work per beat by making use of Starling s formula (19)

$$W = QR + \frac{wV^2}{2g}$$

in which W equals the work done per beat, Q equals the volume of blood expelled per beat, R equals the mean arterial blood pressure in mm of Hg $\times$ 13 6, V equals the velocity of blood at the aorta, w equals the weight of blood, g equals acceleration due to gravity The last part of the formula, $wV^2/2g$, has been omitted in order to make our results comparable with those of Starr, Collins, and Wood (12) By substituting values in this formula we have calculated the work of the left ventricle per beat The work per beat done by the left ventricle was found less during the period of decreased cardiac output at low metabolic levels than later when the output was greater and the basal metabolic rate normal (Table I) We have related the work per beat to the size of the heart (Figure 7) Starr and his associates (12) have shown that the work of the left ventricle which is maintaining an adequate circulation bears a linear relationship to the size of the heart, and have defined a zone of normal circulatory function In a similar fashion we have plotted cardiac volumes as abscissae and grammeters of work of the left ventricle per beat as ordinates (Figure 7) Three of the patients (Cases 1, 2, 4) fall outside the zone of normal circulatory function, below the line CD and the fourth (Case 3) within the zone, but close to the line CD In short in 3 patients the work of the heart was not commensurate with its size. As the basal metabolic rate rose with administration of thyroid extract, all move up into the zone of normal circulatory function and closer to the best line AB indicating improved cardiac function

SUMMARY

In the presence of myxedema the cardiac output per minute and per beat are

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[4] " Enlargement " is used without making a distinction between hypertrophy and dilatation.

velocity of blood flow slow, and the heart larger than normal for that individual at a time when the basal metabolic rate is low　Moreover, the work per beat is low and not commensurate with the size of the heart　With the administration of thyroid extract and the increase of the basal metabolic rate to normal levels, the cardiac output increases per minute and per beat　The velocity of the blood flow increases and the heart becomes smaller　The situation is then a reversible one In the myxedematous state the arteriovenous oxygen difference is increased　There is present apparently a defect in the maintenance of the circulation since the circulation rate is slowed to such an extent that it is inadequate even to the decreased tissue requirements for oxygen　It has to be met by encroachment upon the arteriovenous oxygen difference　The explanation of this phenomenon is not now at hand, but it has been discussed in the light of Boothby and Rynearson's hypothesis with respect to hyperthyroidism　It has been demonstrated that the lengthening of the circulation time in myxedema bears a linear relationship to the cardiac output per minute as well as to the oxygen consumption, that the arteriovenous oxygen difference has a linear relationship to the oxygen consumption and the basal metabolic rate

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HEAT STROKE CLINICAL AND CHEMICAL OBSERVATIONS ON 44 CASES [1] [2]

By EUGENE B FERRIS, JR., M A. BLANKENHORN HOWARD W ROBINSON, AND GLENN E. CULLEN

(From the Departments of Internal Medicine and Pediatrics College of Medicine University of Cincinnati the Cincinnati General Hospital and the Children s Hospital Research Foundation Cincinnati)

The problem of heat diseases has long been an important one in the tropics, in certain industries and occupations requiring exposure of individuals to high temperatures, and during periods of excessively hot weather in the cities of the United States In spite of the voluminous literature on the subject, there is no unanimity of opinion regarding the predisposing and precipitating factors which bring about such reactions

Three fairly distinct clinical syndromes may occur as the result of an excessively high environmental temperature. These are heat cramps, heat exhaustion, and heat stroke. The syndrome of *heat cramps* has long been known among workers in hot environments In addition to severe muscular cramps, these patients sweat profusely and have a normal temperature The work of Edsall (1), Moss (2), Haldane (3), Glover (4), Talbott and his coworkers (5, 6), and others (7) suggests that this syndrome results primarily from an excessive loss of electrolytes, namely sodium chloride, in the sweat The symptoms can be relieved or prevented by the administration of sodium chloride, and the mortality is negligible. The syndrome of *heat exhaustion* is characterized by profuse perspiration, pallor of the skin and low blood pressure—manifestations of peripheral circulatory collapse. The temperature may be subnormal, normal, or slightly elevated The symptoms are of a syncopal nature, namely, weakness, dizziness, and sometimes fainting Nausea and vomiting may occur As a rule heat exhaustion is not a serious condition Recovery is rapid and the mortality low *Heat stroke*, on the other hand, is a most serious condition, having a mor-

tality which ranges from 10 to 80 per cent (8, 9) The syndrome is characterized by an extremely high body temperature and profound coma Sun stroke, heat collapse, thermal fever, and heat hyperpyrexia are terms used to describe this condition, the most outstanding feature of which is a high body temperature Heat stroke may be preceded by symptoms of heat exhaustion or it may develop suddenly, without warning to the victim It is probably the cause of the majority of deaths attributed primarily to excessively hot weather

Various observers differ regarding the relative importance of the various factors which, in addition to the high environmental temperature, may be responsible for the breakdown in the heat regulatory mechanism in heat stroke Many observers (2, 3, 10, 11, 12, 13, 14), have stressed the importance of a high humidity Sayers and Davenport (10) and Adolph and Fulton (11) studied its effect by producing moderate elevations of body temperature in humans in a hot, humid environment. Alcoholism (8 10, 12, 13) and old age (10, 13 15) have been demonstrated to be important contributory factors in the development of heat stroke Metropolitan Life Insurance statistics show that 75 per cent of the deaths resulting from heat stroke occur in patients over 60 years of age (16) Since the chief means of heat dissipation are radiation, conduction and convection, and evaporation from the skin, various explanations involving the breakdown of these mechanisms have been proposed as the primary cause for heat stroke. Circulatory failure (11 17, 18 19) acidosis (20), "fatigue" of the sweat glands (21), the influence of the sun on the heat regulatory center (22 23), and increased body metabolism (15) have each been held responsible for the breakdown in the heat

regulatory mechanism and for the clinical mani-
festations

There is also a difference of opinion in the
literature concerning certain of the clinical mani-
festations, such as the state of the peripheral
circulation, cardiac function and function of the
sweat glands, which might bear on the patho-
genesis as well as therapy in this condition   A
number of observers have stressed peripheral cir-
culatory failure or congestive heart failure as
important clinical manifestations of heat stroke
(13, 14, 22, 24, 28), giving the impression that
pulmonary edema, cardiac failure, and shock are
common   In a series of 64 cases reported by
Gauss and Meyer (8) failure of the circulation
was not a prominent feature   While several ob-
servers (7, 8, 13, 21, 22, 23), report that sweat-
ing is typically absent in heat stroke, others (9,
13 24), state that sweating may occur

During two severe heat waves in the summer
of 1936, 44 patients suffering from heat stroke
were admitted to the Medical Service of the Cin-
cinnati General Hospital   We think that the re-
sults of our studies on these patients should
clarify certain conflicting ideas regarding the
pathogenesis, clinical manifestations, and treat-
ment of heat stroke

METHOD OF STUDY

Only those patients in whom a definite diagnosis of
heat stroke (hyperpyrexia) could be made were selected
for this study   The diagnosis was not difficult in those
having rectal temperatures above 106° F   For 9 patients
who had admission temperatures ranging from 104 to
106 the diagnosis depended upon the elimination of other
possible causes of an elevated temperature, and upon the
subsequent course

Because the majority of the patients were admitted
over a very short period of time our facilities were
greatly overtaxed and complete studies could not be made
on all   However 39 patients who lived long enough to
be examined were given routine physical examinations
All had taken for routine red blood cell count
hemoglobin determination white blood cell count differ-
ential smear and blood before treatment was in-
stituted   The rectal temperature of all patients was followed
routinely   Rectal temperatures were recorded
at frequent intervals and were calibrated only
after the temperature approximated closely
that registered   112° F or above

The clinical and blood
studies were carried out on admission and re-

peated immediately following the reduction in temperature
by ice water baths but before any other therapy was in
stituted   Control studies on those patients who survived
were made just previous to discharge and at least 2 days
following any parenteral administration of fluid or salt.
In addition to a routine examination, particular attention
was paid to the arterial blood pressure, respiration, pulse
skin color, mental state, lung signs, presence or absence of
edema, and venous blood pressure of each patient in this
group   Blood samples were obtained under oil from the
femoral artery and femoral vein and were used for as
many of the following determinations as possible   non
protein nitrogen, total protein, A/G ratio, sodium chlo
ride, sodium, potassium, cholesterol, hemoglobin, hema
tocrit, red blood cell count, carbon dioxide content, and
oxygen content. The chloride concentration of the initial
specimen of urine was determined in 2 patients   The
clotting time of venous blood obtained at the time of ad
mission was determined in 5 cases   The pressure in the
femoral vein was obtained by the direct method of Moritz
and Tabora (25) in 12 cases   Clotting time was de
termined simultaneously by the tube and capillary methods

Chlorides were determined by the method of Van Slyke
(26)   Oxygen and carbon dioxide contents of the oxa-
lated blood, taken with the precautions outlined by
Austin, Cullen, Hastings, McLean, Peters, and Van Slyke
(27), were made by the gasometric methods described
by Peters and Van Slyke (28), employing the Van
Slyke-Neill manometric apparatus   The oxygen capac-
ity values were calculated, using the factor of 1.34 cc.
per gram of hemoglobin, from hemoglobin determina
tions that were obtained by the colorimetric carbon mon
oxide method of Palmer (29)   A Wratten filter, Num
ber 74, was employed with the Duboscq colorimeter   The
percentage of red blood cell volume was determined by
the method of Guest and Siler (30)   The serum protein
values are based on nitrogen determinations by the micro-
Kjeldahl method   In the fractionation of the serum pro-
tein, the Howe method (31) with the precipitation of the
globulin by 22 per cent sodium sulfate solution was used.
The details of the micro-Kjeldahl apparatus, digestion,
and distillation are described by Robinson, Price and Cul
len (32)   Serum sodium was determined by the uranyl
zinc acetate method of Butler and Tuthill (33), and
serum potassium by the method of Shohl and Bennett
(34)

Because of the temporary confusion which resulted
from the sudden appearance of so many critically ill pa
tients on the medical wards a standard routine of treat-
ment could not be immediately formulated. Therefore,
an opportunity to observe the effects of several recog-
nized forms of treatment was presented   The 9 patients
whose rectal temperatures were 106° F or below wer
either treated initially with cold sheets and fans or were
given no specific temperature lowering treatment   Since
these patients were conscious and rational and were able
to take fluids by mouth a container of water having 4
grams of sodium chloride per quart was placed at the
bedsides for them to drink as desired   Two of the

severely ill patients [a] also were treated with cold sheets and fans. This procedure consisted in wrapping the patients in cold wet sheets and facilitating evaporation by blowing air over the sheets and at the same time massaging the skin through the sheets. Twenty five of the severely ill patients were immersed in a tub full of ice water and the skin was rubbed vigorously until the temperature was approximately 100 to 102° F Following the reduction in temperature, 23 of this severely ill group were given physiological saline parenterally in amounts ranging from 2000 to 4000 cc. daily until they either died or recovered sufficiently to take fluids by mouth. The remaining 2 patients of this group of 25 were given no fluids until they recovered sufficiently to take them by mouth.

One patient was administered 1500 cc. of physiological saline intravenously before physical measures calculated to lower the temperature were instituted. One patient was subjected to a venesection of 500 cc. One severely ill patient was given no specific treatment because he improved before cooling measures could be used. Five patients died before any therapy could be instituted.

A history was obtained from the patients who recovered and from the family and friends of all patients In addition to the character and duration of symptoms leading up to the heat stroke, they were questioned particularly regarding habits environment, occupation physical exertion, exposure to sun, other diseases and degree of sweating before the onset In addition, a social service worker from the City Health Department inspected the home and environment of most of the cases.

RESULTS

Environmental and weather conditions The official temperatures which were recorded during the July 1936 heat wave are listed in Table I These were obtained by the United States Weather Bureau at the Clifton Station The severity of this heat wave is indicated by the fact that for a period of 8 consecutive days the maximum temperature ranged from 102 to 106° F A less severe heat wave of 4 days' duration occurred in August, but since 42 of the 44 patients included in this study were stricken during the July heat wave we shall limit the description of weather conditions to this period We have no exact knowledge of the temperature of the environment in which the individual patients lived, but it is certain that their actual environment was considerably hotter than the official temperature

TABLE I

Official weather reports obtained from the Clifton Weather Observatory during the July 1936 heat wave

Date	Official temperature					Relative humidity			Rainfall	Number of patients admitted
July	Maximum	Minimum	Mean	8 p.m.	Midnight	8 a.m.	Noon	8 p.m.		
	F	F	F	F	F	per cent	per cent	per cent	inches	
8	103	70	86	90	83	68	31	43	0	0
9	102	75	89	91	85	70	36	45	0	0
10	105	78	92	93	81	59	26	44	0	4
11	104	72	88	80*	79	77	41	63	0.65	0
12	105	74	90	96	88	68	36	33	0	8
13	102	79	90	96	85	63	31	32	0	7
14	106	75	90	96	80	64	28	35	0	7
15	104	78	91	82	78	68	35	55	0	14
16	91	74	82	85	81	80	66	66	0	2
17	98	71	84	92	81	58	16	23	0	1
Average July 8 to 15	104	75	89.3	94	85	65.3	33	45		
Average for month exclusive July 8 to 15	89 4	63.5	79	79.5	75	72.2	45 4	49		

* With rainfall, the temperature fell from 104° at 4 p m to 80 at 5 p m. on July 11 The night temperature averages do not include temperatures on this date or on the evening of July 15 when the heat wave subsided

at the Clifton Station which is situated on one of the hills which surround the more thickly populated " basin " section of Cincinnati The Bureau also maintains a station on top of the government building approximately 75 feet above street level in downtown Cincinnati, which is situated on low, level ground along the Ohio River basin This region includes the business and crowded tenement districts of the city The average mean temperature during the period under discussion was 2° F higher in the downtown district The daily maximum temperature was identical at the two stations but the night temperature was considerably higher in the " basin "

An investigation of the homes of patients who developed heat stroke indicate that the majority lived under conditions which favored an excessively warm environment, namely, the majority lived on top floors of brick buildings covered with flat roofs [4] It is of interest that 91 per cent of the patients resided in densely settled regions of the city

The relative humidity as determined 3 times daily by the Weather Bureau in Clifton is listed

n Table I The average humidity, as one would expect, was considerably less during the heat wave than the average for the remainder of the month

Direct exposure to the rays of the sun did not appear to be an important factor, as only 10 patients had been exposed for a significant period of time Likewise, physical exertion appeared of little importance Only 9 patients were doing work which at most required moderate exercise at the time of or preceding their collapse Thirteen of the patients were employed and working during the heat wave, but none was engaged in occupations which required exposure to an environment any warmer than that encountered by the average person

The daily number of admissions of patients suffering from heat stroke is tabulated in Table I There were no admissions until the third day of the heat wave, and thereafter, with the exception of July 11, the daily admissions rose progressively for the duration of the heat wave The lack of admissions on July 11 was probably owing to the fact that coincidently with a rainstorm in the early afternoon the temperature fell rapidly and remained near 80° F for the remainder of the day and night It is significant that most of the patients entered the hospital in the afternoon or evening

Other predisposing factors The age of the patients varied from 25 to 90 years However, only 7 of them were less than 50 years of age This distribution of ages is shown in Figure 1 It is evident that the greatest frequency of age distribution is in the period of 60 to 70 years

Alcoholic beverages appeared to play a significant role in the development of heat stroke in a number of patients We were able to get reliable information concerning the drinking habits of 38 patients Of these, 17 (44 per cent) had consumed significant amounts of beer or whiskey on the day of their collapse and in many cases for several preceding days However, only 2 of these patients were considered to be chronic drinkers and none showed definite evidence of deficiency disease Seven additional patients had consumed amounts of alcohol insufficient to be considered significant Fourteen patients, chiefly women, denied consumption of alcohol

As the age of the patients would indicate, the
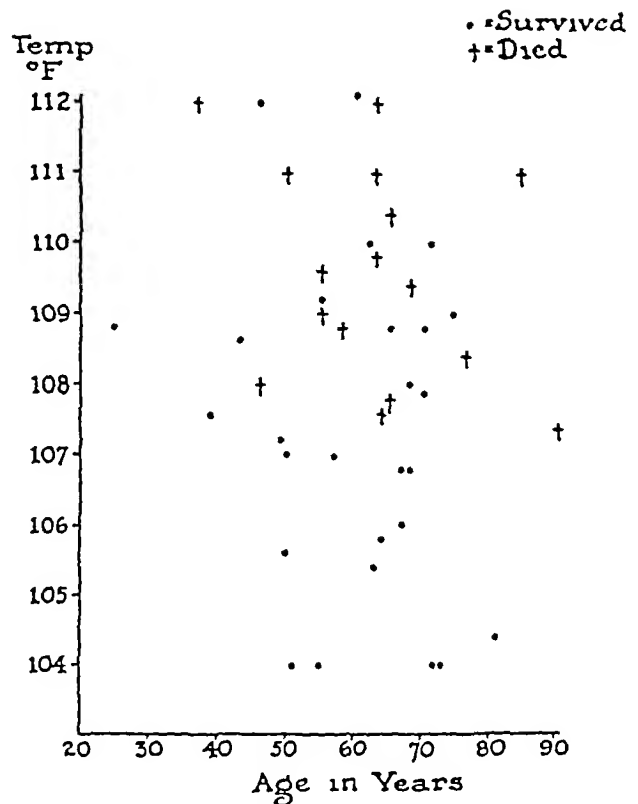
FIG 1 A COMPOSITE CHART INDICATING THE AGE, ADMISSION TEMPERATURE, AND OUTCOME IN 44 CASES OF HEAT STROKE.

Most of the patients were over 50 years of age None of the patients having temperatures below 107° F died In the patients having temperatures above 107° F, there is no clear relationship between temperature and mortality or between age and mortality

majority presented clinical evidence of degenerative vascular disease Eight gave a history of symptoms suggesting early congestive failure, of these, 2 had auricular fibrillation and 2 gave a history suggestive of coronary artery disease Benign arterial hypertension was present in 10 patients Two suffered from degenerative disease of the brain Of the 3 patients who were less than 40 years of age, 2 were alcoholic addicts, and the other suffered from an acute respiratory infection, postpartum anemia, and malnutrition This relationship between heat stroke and disease has been noted previously (35) The sex incidence was of no significance, 27 were males and 17 females The racial incidence may be significant, 37 were white and 7 colored, a ratio of 5 to 1, whereas the ratio of white to colored patients admitted to the hospital from the densely

populated regions of the city during the summer of 1936 was roughly 3 to 2

Prodromal symptoms An adequate history of symptoms preceding the onset of heat stroke was obtained from 24 patients Fourteen patients suddenly collapsed and lost consciousness Of the 14 patients 5 had suffered from weakness, dizziness, nausea, and occasional attacks of fainting over a period of 2 to 3 days preceding their collapse, 5 had noticed slight headache or weakness and a feeling of excessive heat, and 6 had had no premonitory symptoms whatsoever The onset was gradual in the 9 patients who suffered from mild heat stroke and was accompanied by a feeling of excessive heat. The onset was gradual in only 2 of the patients who were affected severely A history of typical muscle cramps was obtained from only 1 patient, however, a number had suffered from attacks of abdominal pain previous to the onset of the stroke.

An attempt was made to ascertain from the histories whether or not these patients had noted any abnormality in their sweating habits during the period of hot weather preceding their illness and also near the time of onset of their symptoms In 21 patients who could give information on the subject, sweating did not appear to be significantly altered during the hot weather previous to the onset of their illness Five thought that they had sweat excessively 3 less than usual, and 13 had perspired normally under the circumstances *However, a significant number noted cessation of sweat just previous to the onset of heat stroke* Seventeen patients noted and volunteered the information that they ceased sweating at this time. None of the patients gave a history of excessive sweating at the onset.

Clinical manifestations

Observations during the acute attack The more important clinical manifestations which were observed in this study are tabulated in Table II Since the syndrome of heat stroke has been frequently described (8 12, 20, 21), we shall limit this report largely to those manifestations which we believe to have a bearing on the mechanism of this condition or about which there is some confusion

The most outstanding findings in this group of patients were an abnormally high body temperature, coma, and a dry skin Depending on the body temperature, these patients could be divided into 2 distinct groups Nine of the patients had temperatures of 106° F or less These patients were conscious and did not appear to be extremely ill The remaining 35 patients had temperatures ranging from 106 8 to 112° F, were either unconscious or stuporous, and were classified as being severely ill The distribution of the temperatures is shown graphically in Figure 1

The *absence of sweat* was a finding common to all 44 patients and was the most characteristic feature. The skin was very hot, dry, and flushed In 23 patients a characteristic maculopapular skin rash was present over the body being most marked over the chest, abdomen, and back. This eruption was fiery red in color and in many areas was purpuric On admission most of the more severely affected patients who were in coma showed other evidence of a depressed nervous system, the muscles were flaccid, respirations were rapid and deep, tendon reflexes were diminished, and the patients were incontinent of feces The 9 patients less severely affected presented no abnormal neurological findings except that several were mentally confused Respirations were increased in rate and depth but were not labored

Because both cardiac and peripheral circulatory failure have been described as characteristic features of heat stroke and also have been held responsible for this condition, we shall describe in more detail the changes which were observed in the cardiovascular system These findings are listed in Tables II and III In no instance was there evidence of definite congestive failure, as peripheral edema, venous engorgement, and orthopnea were absent The more severely affected patients showed evidence of increased bronchial secretion in that large bronchial and tracheal rales could be heard However, parenchymal basal rales were present to a significant degree in only 7 patients, most of whom had a previous history of mild congestive failure Pulmonary edema was evident in only 1 obviously moribund patient. The absence of a significant degree of congestive failure was further evident in those patients studied more extensively (Table III) in whom venous pressure and arterial oxygen saturation were relatively normal Furthermore, it is significant that with the exception of basal rales,

The venous pressure of 12 of the more severely ill patients ranged from 2 0 to 12 cm saline, in most instances being at the upper limits of normal The skin was hot and hyperemic The difference in oxygen content between the femoral arterial and venous blood ranged from 3 1 to 10 5 volumes per cent in 6 patients, the average being 6 4 These figures do not indicate a great retardation in peripheral blood flow when one considers the greatly increased tissue metabolism which must be present with such a high body temperature In the absence of congestive failure, the presence of a rapid full pulse, high pulse pressure, hyperemic skin without cyanosis, and a high normal venous pressure in the majority of patients suggests that in most instances the peripheral blood flow rather than being decreased was actually considerably faster than normal

Observations following reduction in body temperature Twenty-five of the more severely affected patients were immersed in ice water immediately after admission, thereby causing a rapid reduction in the body temperature and a dramatic change in the clinical appearance as the falling temperature reached 107 to 106° F At this point the patients, who were previously flaccid and unconscious, became rigid and struggled so violently that considerable effort was necessary to prevent their escaping from the tub or injuring themselves Several of the patients regained consciousness at this point and many others muttered incoherently Goose-flesh appeared over the skin The patients were removed from the tub when their temperatures had reached 102 5 to 99° F The observations noted at this time are listed in Table III There was a striking decrease in the respiratory rate and pulse rate The blood pressure approached a more normal level in most instances, namely, the pulse pressure became less and the systolic pressure fell The blood pressure also approached normal in several of the patients in whom it had previously been low The majority of the severely ill patients remained delirious and required restraint for from 4 to 12 hours after being cooled One patient subsequently developed delirium tremens and 2 patients remained in coma until death supervened The temperature remained unstable for from 3 to 12 days ranging from 97 to 103° F and was usually elevated The majority of

the patients did not sweat until several days after admission In 2 patients sweating was observed to occur simultaneously with the return of the temperature to normal

The chemistry of the blood

Blood concentration In Table III are tabulated values for protein, hemoglobin, and cell volume percentage In most instances the serum protein and hematocrit values were greater than the control values of samples obtained after clinical recovery and indicate that hemoconcentration was present (Table III) The degree of blood concentration was much less than that reported by Talbott *et al* (6) in patients with severe heat cramps who, in contrast with our patients, were conscious and had normal body temperature It is of interest that in 7 instances blood samples taken from patients immediately after therapeutic reduction in temperature showed evidence of greater concentration than on admission, in spite of the fact that the patient's condition had improved greatly

In 8 cases albumin was determined, and in all 8 there was no evidence of the high globulin, low albumin ratio reported for heat cramps Moreover, the high level of albumin in those patients with the high total protein level is much greater evidence for a concentrated blood owing to loss of fluid than is the total protein level alone

Sodium and chloride concentration in the serum In Table III are included the values for chloride level in serum from 18 patients and for sodium in 7 patients It is at once evident that the chloride level on admission varies to both sides of the normal values of 98 to 108 m eq per liter (557 to 632 mgm per cent sodium chloride) In only 1 case (Case 26) was the chloride at the extremely low level found by Talbott and others in severe heat cramps and in only 3 other cases was the chloride level below the round figure of 96 m eq In 3 patients the chlorides were much above the normal level Of these, the high values in Cases 27 and 28 were possibly due to concentration of blood serum as evidenced by the high protein level There is no evidence of such concentration in Case 11 which is unusual in that the chloride level on admission was high without elevation either of albumin or total protein, although there was evidence of a concentration of

hemoglobin After the initial tubbing, although it is evident from the protein and hemoglobin values that there was a concentration of blood and serum, the chloride fell

In the 1 case (Case 26) in which the chloride was already extremely low, both the chloride and sodium concentration rose during the half-hour of tubbing

The sodium values ran fairly parallel with the chlorides Potassium was determined in only two cases Case 10, 3 52 p.m = 3 8 m eq, and Case 32, 8 30 a m = 2.2 m eq

All these values, taken together, make it appear to us that the situation with regard to the electrolytes in this type of heat stroke is entirely different from that of heat cramps, and that in the present case the changes in electrolytes are coincidental rather than causative

The acid base balance The total carbon dioxide content of both femoral arterial and venous blood was definitely low, although in no case was the alkali reserve lowered sufficiently to account for the coma Immediately after the initial tubbing and reduction of body temperature in 5 cases there was little change in carbon dioxide content, in 3 there was a further definite decrease of 4 to 5 volumes per cent carbon dioxide in spite of marked clinical improvement. (Note that these values are for whole blood which is always lower than serum) Two patients in whom an increased blood concentration occurred coincidentally with cooling likewise developed a more severe acidosis Since the reduction in body temperature was accompanied by considerable muscular exercise incident to struggling the increased oxygen debt and lactic acid accumulation caused thereby may account for this lowering

Unfortunately, determinations of pH and lactic acid were not obtained in this study, but analysis of the data furnished a fairly clear picture of the acid base condition There is no consistent shift of chloride in relation to serum protein, blood carbon dioxide content, or total sodium which would indicate an important alteration in the total electrolyte balance The variations in total carbon dioxide content are well outside the possible changes that might be a result of the alteration in blood temperature (35) or to overventilation We conclude, therefore, that the acidosis is due to the accumulation of non volatile metabolic acids, probably lactic and that it is secondary to the high body temperature. This interpretation is in agreement with the work of Hall and Wakefield (36), who produced hyperpyrexia in dogs and found a great increase in the lactic acid and reduction of the pH of the blood

Oxygen content The oxygen content of the arterial blood in 7 patients at the time of admission ranged from 13 5 to 17.9 volumes per cent and averaged 16.9 volumes per cent The average arterial oxygen content of blood obtained from the same patients at the time of discharge was 16 7 volumes per cent The oxygen content of blood obtained from the femoral vein varied from 7.2 to 13 0 volumes per cent The arteriovenous oxygen difference varied greatly, as Table III indicates Because the tissue metabolism is probably greatly increased in this condition and is probably subject to great changes under the conditions of these studies the A–V oxygen difference can not be used to estimate the relative blood flow in the leg It is of interest, however, that the arterial oxygen content was approximately normal in these patients

Other blood findings The clotting time was normal in 5 of the severely affected patients whose skin rash was purpuric in nature No increased tendency to bleed from skin puncture wounds was noted

Urine chloride concentration The concentrations of urinary chloride in admission specimens were 35 and 45 m eq for Cases 43 and 44 These values are low but not as low as those found by Talbott in heat cramps

Treatment

Cold sheets and fans The use of cold sheets and fans to facilitate evaporation and thus to lower the body temperature has been advocated by many writers (13, 20 24, 37) Ten patients were treated by this method Of these patients, 8 were classified as mild cases in that they were conscious and had temperatures below 105° F , and as a result of this treatment the temperature of each gradually fell and the 8 patients recovered The result of this form of treatment in Cases 3 and 37 who were in coma and had greatly elevated temperatures, was not satisfactory in that the procedure failed to lower the body temperature and both patients died

Ice water tubbing Twenty-five patients, all classified as severely ill, were subjected to this form of treatment The body temperature was reduced to below 102° F in from 9 to 40 minutes The method proved to be very effective in lowering the body temperature rapidly and was not associated with any untoward effects except in 1 patient, whose temperature fell to 99° F before he was removed from the tub The temperature of this patient subsequently fell to 96° F and he developed circulatory collapse Although hot blankets effectually elevated the temperature, the patient subsequently died Quite possibly such excessive cooling contributed to his death

Of the 25 patients treated in this manner 8 died, a mortality rate of 32 per cent, which seems low considering the fact that all 25 patients were severely ill

General measures After adequate reduction in body temperature, the patients were watched carefully for unusual changes in body temperature, and ice packs, wet sheets with fans, or hot water bottles were applied until the temperature became stabilized

Fluids averaging a total of 4000 cc per day and sodium chloride averaging 16 grams per day were given by mouth to those patients who were sufficiently rational to drink and by the subcutaneous and intravenous routes to the others Fluids and salt were withheld from 2 of the more severely ill patients until they were able to take them by mouth a period of approximately 6 hours The administration of fluids and salt had no obvious influence on the course of these patients' illnesses The 2 from whom fluids were withheld recovered, and their clinical course was no different from that of the other patients One severely ill patient (Case 31) was given 2000 cc of cold physiological saline intravenously as a usual treatment This procedure did not lower the body temperature after 30 minutes, and the patient subsequently died One patient was subjected to a venesection and promptly died

Of the 44 patients admitted to the ... 17 or 39 per cent, died Of the 39 ... 12 or 30 per cent, died The ... to be good in those patients

who were conscious and had temperatures below 107° F, since none of these died

Pathological findings Postmortem examinations were performed on 12 patients The findings were of interest chiefly in that the majority of patients showed evidence of degenerative vascular disease Bronchopneumonia was thought to have caused death in 2 cases The others appeared to have died of heat stroke, as no anatomical cause for death could be found The postmortem studies added no information to the pathology of heat stroke other than that already described (38)

DISCUSSION

The primary cause of the breakdown in the heat regulatory mechanism during exposure to high environmental temperatures has long been a controversial subject The various mechanisms which the body has for dissipation of heat are well known The mechanisms which dissipate the major portion of heat are first, radiation, conduction, and convection from the skin, and second, evaporation from the skin and, to some extent, through expired air The first mechanism plays a major rôle in heat dissipation under normal conditions of body heat production when the environmental temperature is below that of the body The second mechanism becomes the most important factor under the environmental conditions of this study where the atmospheric temperature approached or exceeded that of the body Under such conditions as those found during the heat waves of 1936, it is obvious that little heat can be dissipated by means of radiation and that the major portion must be lost through the process of evaporation That the body can tolerate extremely high environmental temperatures for a short period of time is borne out by investigation of conditions in various occupations such as those of steel workers (39) and stokers (40) It is important that such workers live in a considerably cooler environment in between such daily exposures, when presumably the body has a chance to compensate for the temporary overactivity of its heat dissipation mechanisms Under such extreme conditions heat cramps may be prevalent unless measures are taken to replace the chlorides and water lost in the sweat

Our findings suggest that the cumulative effect of prolonged exposure to high temperatures is the important factor in the etiology of heat stroke in our patients. The patients did not develop heat stroke until the third day of the heat wave, and with the exception of 1 day the incidence of daily admissions rose steadily until the heat wave was over. This latent period has been repeatedly mentioned in the literature. As indicated in Table I, the apparent reason for the lack of admissions on this one day was a sudden drop in temperature which accompanied a rainstorm. On the other days the official temperature remained very high, not only throughout the day but also through most of the night. The fact that the official mean temperature was 2° F higher in the downtown section of the city than in the outlying Clifton district may be related to the observation that 91 per cent of our patients resided in densely settled regions. Furthermore, considering their environment, it is reasonable to assume that the majority of the patients studied were exposed to considerably greater temperatures throughout the night than the official report would indicate. The relatively low humidity present with these heat waves when the wet bulb temperature never exceeded 76° F, and also the lack of excessive sweating and of premonitory symptoms, such as occurs in experiments carried out in atmospheres of high humidity, are facts which suggest that humidity did not play an important role in the production of heat stroke in these patients. Therefore, as far as the weather is concerned, the most important factor in this study was the exposure of patients to excessively high temperatures over a prolonged period.

Our results indicate a definite relationship between a sudden alteration in the sweating mechanism and high or unstable body temperature. In most instances cessation of sweat occurred acutely just preceding the actual heat stroke. Since most of the patients collapsed during periods when the environmental temperature exceeded that of the body, it is to be expected that the body temperature should have risen when sweating ceased. The observation that on admission none of the patients showed evidence of sweating and that the temperature of several patients remained unstable until sweating returned corroborates the history with regard to the im-

portance of sweating. The exact mechanism which brought about the sudden cessation of sweating in these patients is not clear. It has been suggested that the sweat glands become fatigued because of overwork or because of dehydration or low body chloride. According to the history of these patients, they had perspired no more than usual during the days preceding their collapse, and as a group they had lost little or no excess of fluids or chlorides by other ways. Although blood studies indicated the presence of moderate dehydration, it was of much less extent than that seen in other conditions, such as heat cramps where sweating is profuse but in which there is no alteration in temperature. Likewise, the relatively normal blood chlorides which we found to be present did not indicate excessive loss through the sweat, nor was the degree of sweating or the clinical course strikingly influenced in these patients by the administration of sodium chloride or fluids.

Failure of the circulation has also been held to be a precipitating factor in the breakdown of heat regulation. In those patients whose collapse was preceded by symptoms of heat exhaustion such as dizziness, weakness and fainting attacks, it might be argued that a deficient peripheral circulation did exist while blood was pooled in the periphery. However, an appreciable number of patients had no premonitory symptoms whatsoever, and, of more importance, the usual case of heat stroke showed no evidence of circulatory or of definite cardiac failure even when there was a preexisting history of cardiac symptoms and objective evidence of organic heart disease. Thus, although it is quite reasonable to assume that a high body temperature may be brought about through the failure of a deficient peripheral circulation to bring heat to the surface—and such has been shown to occur to a slight degree in cardiac failure (41)—the suddenness of onset and the lack of evidence of deficient circulation in our patients precludes this mechanism as being an important factor.

Besides environmental conditions, the most outstanding predisposing causes for the development of heat stroke in this group of patients were old age and the degenerative diseases which commonly accompany senility. Since the majority of these patients were not engaged in activities which

would increase their body heat production, and since there is evidence that such elderly patients have a decreased tissue metabolism rather than an increased one, it is probable that excessive heat production is not a factor, but that their mechanism for heat dissipation is less effective than that of more normal individuals and can not compensate for as long a time when the person is exposed to a high environmental temperature

The only other predisposing factor of great importance was the association of the ingestion of alcoholic beverages with the onset of heat stroke Inasmuch as many of these patients were not chronic alcoholic addicts and gave no history of poor diet, and since the onset of heat stroke was closely related in time to the ingestion of alcohol, it seems probable that the heat stroke in such patients was precipitated by the effect of alcohol itself rather than by such secondary factors as malnutrition and vitamin deficiency, which are frequently associated with chronic alcoholism Alcohol is known to depress vasomotor reflexes (42, 43), and to produce dilatation of the peripheral vessels (39, 43, 44, 45), thus increasing the loss of heat from the body and resulting in a fall in body temperature The fall in body temperature has occurred, however, under conditions where the environmental temperature was lower than that of the body Conversely, in an environmental temperature higher than the body, these same effects would serve rather to promote absorption of heat and thus add to the burden of dissipating heat through evaporation This reasoning is borne out by the work of Barbour and Bourne (45) who showed that ether, which acts similarly to alcohol, causes dogs to become poikilothermic It has also been shown that alcohol stimulates the sweating mechanism through its central action (46) Whether or not such an added stimulus to an already overworked sweating mechanism might be in part responsible for its failure remains to be demonstrated

Our observations suggest that the clinical findings and chemical changes in the blood are the result or rather than the cause of the high body temperature The dramatic improvement in many patients following the reduction of temperature without other treatment and without a sudden change in the blood chemistry suggests that the coma is related closely to the high temperature Likewise, the respiratory rate decreased markedly without change in the carbon dioxide content of the blood, again suggesting that the increased respirations are due in part to high temperature and not altogether to acidosis The moderate degree of blood concentration likewise did not appear to be an important factor in the symptomatology or etiology of the condition, as much more marked dehydration and blood concentration may occur without any of the symptoms of heat stroke The sodium and chloride level of the blood in these patients was not significantly altered and these findings, together with the lack of evidence of excessive chloride loss previously, the presence of chloride in the urine, and the lack of effect of chloride administration on the clinical course, all serve to eliminate low sodium chloride concentration as an important factor in heat stroke Normal blood chlorides in heat stroke have also been reported recently by Heilman and Montgomery (47)

Our observations are in agreement with those of most observers that in heat stroke the most important therapeutic measure is to lower the body temperature promptly and to maintain it at an approximately normal figure In those patients who are conscious and have temperatures below 106° F , the temperature may be lowered best by mild measures, such as wet sheets and fans or ice packs In the severely affected patients, however, such measures did not lower the temperature effectively in our hands, and immersion of the patients in ice water was the most efficient method Vigorous massage of the skin is of importance in any type of hydrotherapy Since the patients are moderately dehydrated, administration of fluids is indicated but is of secondary importance We found no indication for the routine use of cardiac and circulatory "stimulants"

Since certain theoretical objections have been raised in regard to the form of hydrotherapy which should be used in this condition, the subject deserves further comment Water sprays to the body and fans to facilitate evaporation have been advocated as being preferable to immersion in ice water because evaporation of a gram of water will remove 590 calories, while the melting of a gram of ice will remove only 80 calories, and further, that immersion of the body in ice water is

not only a shocking procedure but also will diminish the blood flow to the skin (13, 20, 37) The argument that evaporation of water on the skin is more efficient in removing heat than immersion in ice water does not agree with our clinical results nor is it tenable on theoretical grounds when an abundance of ice is available Furthermore, the immersion in ice water of patients having body temperatures above 106° F did not produce shock nor did we observe any pallor of the skin or other signs which indicated a decrease in blood flow to the skin

In conclusion it may be well to define what we consider the syndrome of heat stroke to be   It is our feeling that moderate elevation of the body temperature as the result of exposure to heat is in itself not sufficient evidence on which to make the diagnosis of heat stroke   The elevation in body temperature must be accompanied by evidence of a deranged heat regulatory mechanism such as the absence or diminution of sweat, failure of the body temperature to approach a normal level promptly when the patient is removed to a cooler environment and subsequent instability of the body temperature. This derangement of the heat regulatory mechanism is usually brought about as the result of exposure of an especially susceptible group of individuals to a high environmental temperature for several days   The high body temperature, which usually develops in dramatic fashion is the chief cause of the typical clinical manifestations   Profound abnormalities of the peripheral circulation or of the acid base or water balance although rarely encountered in our patients might well occur in patients with heat stroke who, as a result of exposure to a hot moist atmosphere, had sweat profusely, or in whom vomiting or diarrhea had been a prominent feature   Our studies suggest also that similar abnormalities may result if the period of hyperpyrexia is prolonged

SUMMARY

1 Studies on 44 patients suffering from heat stroke are presented

2 Cardiac failure or peripheral circulatory collapse was not evident in the majority of patients

3 The sodium chloride content of the blood was not significantly altered

4 The condition was associated with a moderate acidosis and hemoconcentration

5 The high body temperature appeared to be the chief cause of the symptoms of heat stroke.

6 Old age, degenerative disease, and acute alcoholism are important contributing factors in heat stroke.

7 The onset was precipitated by a diminution or cessation of sweat in the majority of patients

8 Although the primary cause for the sudden cessation of sweat has not been determined it would appear from these studies that loss of chlorides, dehydration, and circulatory failure were not responsible

9 Measures to lower the body temperature promptly are indicated, in our hands ice water tubbing with massage was the most effective method in severe cases

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THE MEASUREMENT OF THE TUBULAR EXCRETORY MASS, EFFECTIVE BLOOD FLOW AND FILTRATION RATE IN THE NORMAL HUMAN KIDNEY

By HOMER W SMITH WILLIAM GOLDRING AND HERBERT CHASIS

(From the Departments of Physiology and Medicine New York University College of Medicine and the Third (New York University) Medical Division of Bellevue Hospital, New York City)

In exploring the normal and abnormal function of the kidney it is desirable to possess, in addition to knowledge of the filtration rate, knowledge of the total mass of functional renal tissue and the rate of blood flow to this tissue In theory, both data can be determined by the clearance method and procedures suitable for these purposes and applicable to man are presented in this paper with the more important physiological considerations upon which these procedures are based. The principles involved in the measurement of the filtration rate and the experimental basis warranting the use of the inulin clearance for this purpose have been reviewed elsewhere (13, 14), and the present discussion will be confined to a description of methods for its routine determination and to the presentation of data on the simultaneous inulin and phenol red clearances in 25 normal subjects

Tubular excretory mass

Recent investigations have shown that the process of tubular excretion in the lower vertebrates is limited by the circumstance that a given mass of renal tissue can transport from blood to urine only a fixed, maximal quantity of a particular solute per unit time (12, 14) It will be shown here that this same limitation applies to the tubular excretion of at least certain substances in man The measurement of this maximal rate of excretion for any one substance constitutes, therefore, a measurement of what we may call the "tubular excretory mass ' of the kidneys

The measurement of this tubular excretory mass may be approached by a brief discussion of the excretion of phenol red, diodrast and hippuran in man, as illustrated in Figures 1 2 and 3 These data are taken from observations in which the simultaneous inulin, phenol red, and diodrast or hippuran clearances were determined in nor-

mal individuals in whom the plasma concentration of inulin and of one other of these solutes was maintained at a low, constant level, while the concentration of the third solute was raised to a high level (Details concerning the composition of the infusions and their administration are given in the protocols Since these clearances are all essentially independent of the rate of urine flow no detailed reference need be made to this factor in the following discussion)

The data represented by the circles in Figures 1 to 3 will be discussed later in the paper, for the
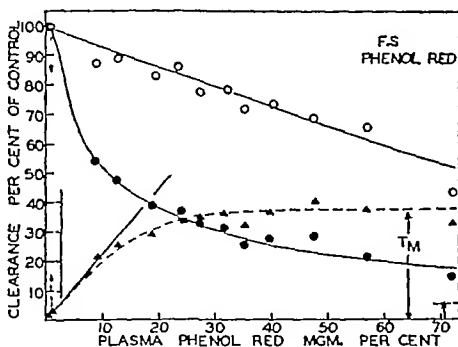
FIG. 1 EFFECT OF ELEVATED PLASMA CONCENTRATION OF PHENOL RED ON THE SELF-CLEARANCE (DOTS) AND ON THE DIODRAST CLEARANCE (CIRCLES) ALL CLEARANCES BEING EXPRESSED IN TERMS OF THE AVERAGE OF TWO CONTROL PERIODS AT LOW PLASMA LEVELS OF PHENOL RED

The control clearances were inulin = 153 phenol red = 456 diodrast = 931 cc. per minute. Observations were made on a falling plasma phenol red curve after the injection of a single large dose and corrected for delay time. The triangles show the rate of tubular excretion of phenol red (mgm. per minute) the ordinates having the values shown outside the frame. T_m is the maximal rate of tubular excretion. The short arrow at the right indicates the minimal (filtration) clearance of phenol red.

263
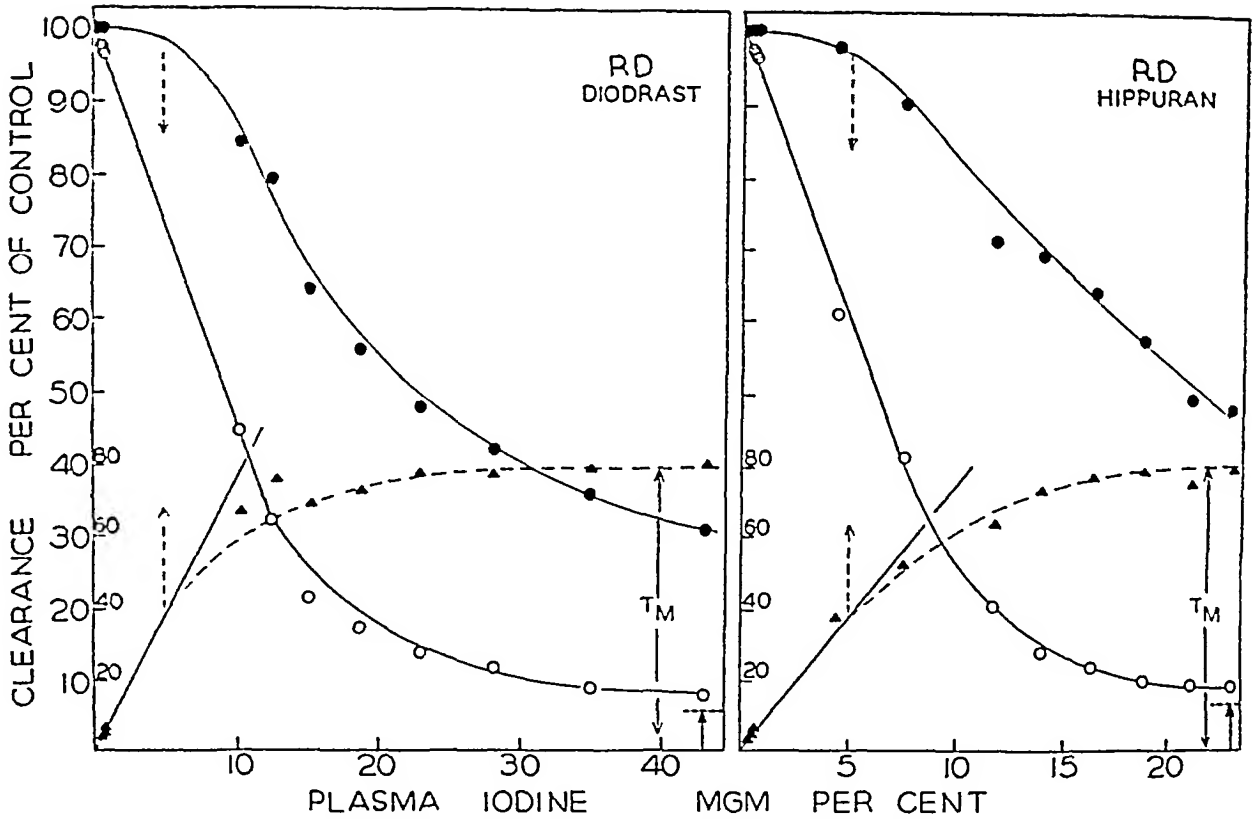
Fig 2 (left) and Fig 3 (right) Effect of Elevated Plasma Concentrations of Diodrast and Hippuran (Both Expressed as Iodine) on the Self-clearance (dots) and on the Phenol Red Clearance (circles) all Clearances being Expressed in Terms of the Average of Three Control Periods at Low Plasma Levels of the Iodine Compounds

The control clearances were (left) inulin = 153, phenol red = 630, diodrast = 867 cc. per minute, (right) inulin = 161 phenol red = 545, hippuran = 803 cc per minute. The observations on diodrast were made on a falling plasma curve after the injection of a large dose of diodrast, those on hippuran were made on a rising plasma curve during the infusion of a strong hippuran solution Both sets of data corrected for delay time.

The triangles show the rate of tubular excretion of the iodine compounds (mgm. iodine per minute) Below about 5 mgm per cent of diodrast- or hippuran-iodine, the rate of tubular excretion of these substances, T, increases in direct proportion to the plasma concentration, in this range, therefore, the clearances are independent of the plasma level Since the diodrast clearance is essentially complete, it affords a measure of the renal blood flow

At higher plasma levels the rate of tubular excretion approaches a maximal value, T_m This maximal rate is of physiological interest since it is independent of glomerular activity and renal blood flow, and is proportional to the number or mass of normal, active excretory tubules T_m is therefore an index of the tubular excretory mass of the kidneys

moment the reader is referred to the data represented by the solid dots and triangles Reference to Figure 1 shows that as the plasma concentration of phenol red is raised the phenol red clearance is depressed Similarly, the diodrast and hippuran clearances are depressed by the elevation of the respective plasma levels of these solutes (Figures 2 and 3) This self-depression of clearance which was first demonstrated for phenol red in man by Goldring Clarke and Smith (8) and for diodrast and hippuran in the

dog by Elsom, Bott, and Shiels (4), is owing to the fact that, in man, as in the other vertebrates, the rate of tubular excretion has an upper limit The rate of tubular excretion, which is shown in the figures by the solid triangles, and which we designate as T, is given by the difference between the total excretion per minute, UV, and the quantity excreted by filtration c,

$$T = UV - PIWF = \left(\frac{X}{I} - WF\right) PI,$$

where U is the concentration of solute per cc. of urine, V the rate of urine formation in cc. per minute, P the quantity of solute in each cc. of plasma, I the concurrent rate of glomerular filtration as measured by the simultaneous plasma inulin clearance, X the plasma clearance of the solute under investigation, W the fraction of water in the plasma and F the fraction of solute which is free in the plasma and therefore available for filtration In this calculation, reference is made to the data on the binding of phenol red hippuran, and diodrast by plasma proteins reported by Smith and Smith (17)

At low concentrations of solute, T increases closely in proportion to plasma concentration, it follows that in this range the clearance has a maximal value and is independent of plasma concentration, as shown by the early horizontal portion of the clearance curve at the left of the vertical dotted arrows But as the plasma concentration increases, more and more of the solute is delivered to the tubules per unit time and T approaches its maximal value, in consequence of this fact an ever decreasing fraction of the total solute in the blood is removed by tubular activity and the clearance, as calculated on the total output in the urine, falls and approaches the filtration rate as a lower limiting value.

The maximal rate of tubular excretion we designate as T_m We may say that the tubules of Subject F S are capable of excreting a maximum of 35.8 mgm of phenol red, and that the renal tubules of Subject R D are capable of excreting a maximum of 80 mgm of diodrast iodine ($= 161$ mgm of diodrast) or 80 mgm of hippuran iodine ($= 206$ mgm of hippuran) per minute when supplied with an abundance of any one of these solutes These values of T_m correspond to 0 10, 0.32, and 0 63 mM per minute of phenol red, diodrast, and hippuran, respectively In three normal subjects hippuran-T_m averages 76, and in five normal subjects diodrast-T_m averages 58 mgm iodine per minute per 1.73 sq m

The datum T_m is independent of the plasma concentration of solute so long as that concentration is adequate at the existing blood flow to supply a sufficient quantity of solute per unit time to maintain the maximal rate of excretion, and it is likewise entirely independent of the renal blood flow so long as that blood flow is adequate,

at the existing plasma concentration, to maintain this maximal rate of excretion It is also entirely independent of glomerular activity If one of the kidneys were removed, T_m would necessarily be cut in half, if part of the excretory tissue were destroyed by disease, whether this destruction consisted of local injury of the tubule cells or obliteration of the circulation so that blood no longer reached normal tubules, T_m would be reduced in proportion to the extent of this destruction If, on the other hand, the glomeruli were to be entirely obliterated without impairment of the circulation to the tubules or injury of the tubule cells, T_m would remain at its normal value. The maximal rate of tubular excretion can be measured just as well in the aglomerular as in the glomerular kidney, and by appropriate standardization it can be expressed as equivalent grams of normal renal tubular tissue (Since these substances are probably excreted by the proximal segment only, T_m is probably an expression of the development and integrity of proximal tubular tissue.)

It is recognized that tubular activity is manifold and consists not only of the excretion of waste products and foreign substances (creatinine, phenol red, diodrast, etc.), but includes chemical transformations (formation of ammonia, hippuric acid, etc) and reabsorptive processes (water, glucose, chloride, etc.) , but it may be assumed that if the integrity of a particular tubule cell is so impaired that it can no longer carry on its normal excretory operations, other operations carried on by that cell will also be impaired This question is subject to experimental investigation since it should be possible to determine whether the capacity to excrete these various substances is impaired differentially during the course of disease, or whether the excretory capacity is impaired *pari passu* with other physiological functions, such as the capacity to reabsorb water, glucose, or other substances Until such differentiation is made we may proceed under the explicitly stated premise that all tubular functions, especially those related to tubular excretion, will vary in a closely parallel manner In this view we speak of T_m, the "tubular excretory mass" measured by any suitable substance, as an index of the residual quantity of functional, tubular tissue.

Effective renal blood flow

It is, in part, in consequence of the fact that the maximal excretory capacity of the tubules differs for different substances that the clearances of these substances, as determined at low plasma levels, may also be different All other factors being equal, one might suppose that the greater the excretory capacity of the tubules for a given solute the more efficiently will that solute be removed from the blood as it passes through the kidneys It is conceivable that for a particular solute, X, the excretory capacity of the tubules might be so highly developed that all the X contained in the renal arterial blood would be removed by the combined activity of the glomeruli and the tubules in one circulation through the kidney If X were neither synthesized nor destroyed by the kidney, and if it were concurrently transferred to the urine, then the clearance of X must in theory be equal to the renal blood flow In short, the renal blood flow constitutes the highest possible figure which the clearance of any substance not synthesized by the renal parenchyma can have It cannot be supposed that all the blood entering the renal artery is distributed to excretory tissue, and insofar as any fraction of the renal arterial blood fails to reach excretory tissue the clearance of X will be proportionately reduced It is therefore advisable to designate the renal blood flow as measured by the clearance of X, the " effective renal blood flow," implying thereby the blood flow to active excretory tissue

To utilize the clearance method for measuring the effective renal blood flow the following points must be considered

a The substance X, should be selected upon the basis of having the highest possible clearance, since the highest clearance must approach most nearly the true blood flow

b The plasma concentration of X must be kept below the level where the clearance is significantly depressed

c It must be ascertained whether other solutes in the plasma, and particularly substances which are normally excreted by the tubules, can interfere with the tubular excretion of X

d It must be shown that X is concurrently transferred to the urine

(Destruction of X by renal tissue and the excretion or storage of X by any other organ do not in theory enter into the problem Synthesis by renal tissue is patently excluded in the case of foreign substances such as are examined here)

The above points will be considered separately

a The relative magnitude of the phenol red, diodrast and hippuran clearances In our search for substances with high renal clearances in man we have examined numerous compounds, among which are several sulphonphthalein derivatives the only one of these approaching phenol red in clearance value being anisole red[1] But since the latter does not appear to be superior, we have limited the present examination to phenol red Among organic iodine compounds we have examined skiodan, iopax, neo-iopax, hippuran, and diodrast A report will be made elsewhere upon the first three, which have low clearances, and the present discussion will be limited to the last two

Typical hippuran and diodrast clearances, together with simultaneous phenol red and inulin clearances, are given in Table I In Table II there are given the average values of a series of observations on normal individuals who were selected by the criteria and examined under the basal conditions described later in this paper

It is evident that both the hippuran and diodrast clearances approach more nearly a complete clearance than does phenol red In the absence of analytical methods which distinguish diodrast from hippuran, it is impossible to determine by simultaneous observations which substance has the higher clearance The average plasma or whole blood clearances, as well as the simultaneous inulin or phenol red clearance ratios, give diodrast a slight advantage, and this substance also has the advantage of being bound to a lesser extent by plasma protein, an important consideration in the calculation of T_m It is possible, however, that our figures are slightly elevated by a vasodilator action, though we have been unable to demonstrate this systematically

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[1] A preparation may be purchased under this name, but Prof W M Clarke advises us that the name may be misleading since the substance it suggests should not behave as one of the ordinary sulphonphthalein indicators We find the commercial compound to be almost identical in its physiological behavior with phenol red

TABLE I

Simultaneous clearances of inulin phenol red and diodrast or hippuran in normal man

Subject	Elapsed time*	Urine flow	Inulin	Phenol red	Iodine	Inulin	Phenol red	Iodine	Phenol red / Inulin	Iodine / Inulin	Phenol red / Iodine / Inulin
	min-utes	cc. per min-ute	mgm. per 100 cc.	mgm. per 100 cc.	mgm. per 100 cc.	cc. per m. per minute	cc. per 1.73 sq m. per minute	cc. per 1.73 sq m. per minute			
						DIODRAST					
J.J	80	Urine discard									
	42	12.3	125	0.93	0.56	130	433	856	3.32	1.96	6.6
	54	9.0	125	1.00	0.61	125	374	744	2.99	2.02	6.0
	65	6.3	127	1.00	0.64	131	383	732	2.96	1.89	5.6
	75	5.3	129	0.95	0.64	125	386	763	3.07	1.99	6.1
F.B.	19	Urine discard									
	41	8.2	100	0.74	0.56	162	495	1070	3.06	2.16	6.6
	52	4.6	100	0.74	0.55	153	478	1003	3.10	.12	6.6
	61	5.2	101	0.73	0.56	151	478	1020	3.15	2.14	6.5
	72	5.6	102	0.73	0.56	144	455	973	3.17	2.14	6.7
						HIPPURAN					
J.J	90	Urine discard									
	42	11.0	118	1.00	0.66	125	440	676	3.52	1.54	5.4
	54	10.0	122	1.01	0.73	114	404	607	3.53	1.50	5.3
	65	10.1	125	1.01	0.78	120	417	602	3.49	1.44	5.0
	75	10.3	126	0.99	0.78	130	464	680	3.56	1.49	5.3
A.M.	97	Urine discard									
	112	3.7	133	0.93	1.08	112	324	533	2.90	1.64	4.7
	127	3.9	133	0.95	1.10	111	329	538	2.97	1.64	4.9
	143	3.4	133	0.92	1.14	108	324	491	3.00	1.52	4.5
	159	2.9	127	0.83	1.10	113	320	540	2.85	1.69	4.8
	175	2.5	123	0.84	1.10	122	339	586	2.80	1.78	4.8

* This column gives the elapsed time from the beginning of the intravenous infusion For details see Methods.

TABLE II

Simultaneous clearances of inulin, phenol red and diodrast or hippuran in normal men *

Subject	Surface area	Number of periods	Plasma	Average plasma iodine	Inulin	Phenol red	Iodine	Filtration fraction X100†	Phenol red / Inulin ‡	Phenol red / Iodine	Minimal renal blood flow (Iodine)
	sq m.		per cent	mgm. per 100 cc.	cc. per 1.73 sq m. per minute	cc. per 1.73 sq m. per minute	cc. per 1.73 sq m. per minute				cc. per min-ute
					DIODRAST						
A.M.	1.59	7	0.50	0.70	122	295	664	18.4	3 17	0.518	1107
J.J	1.73	4	0.53	0.60	128	375	778	16.5	3.30	0.532	1325
H.G.	1.70	4	0.61	0.75	122	375	718	17.0	3.30	0.532	1177
R.D	1.50	4	0.62	0.59	144	498	784	18.4	3.56	0.635	1255
R.D	1.50	4	0.55	0.62	137	571	791	17.3	4.17	0 728	1316
F.B.	1.84	4	0.55	0.56	141	447	956	15.1	3.20	0.453	1728
F.B.	1.84	2	0.54	0.87	144	420	876	16.4	3.14	0.490	1622
V.G.	1.83	3	0.61	0.65	156	543	993	15 7	3.43	0.547	1623
Average					137	463	820	16.7	3.33	0.564	1384
					BUFFURAN						
A.M.	1.59	5	0.54	1.0	120	333	550	21.8	2.93	0 605	1015
J.J	1.73	4	0.53	0.75	122	431	614	19.0	3.53	0.670	1032
H.G.	1.70	6	0.61	0.60	109	394	656	16.4	3.72	0.572	1092
R.D.	1.50	4	0 61	0.90	132	433	770	17 1	3.32	0.560	1092
R.D	1.50	2	0.66	0.75	155	534	803	19.3	3.38	0.653	1316
R.H.	1.74	3	0.58	0.57	108	353	534	20.8	3.37	0.674	904
R.T	1.89	6	0.53	0.75	120	334	630	17.8	2.81	0 477	1307
R.T	1.89	4	0.56	1.0	115	314	634	16.8	3.07	0.433	1222
Average					123	388	655	18.5	3.16	0.534	1109

* All observations with constant plasma levels, as in Table I

† Assuming that the diodrast and hippuran clearances are complete.

‡ Corrected for depression of phenol red clearance according to Figure 4

Landis, Elsom, Bott, and Shiels (10) state that neoskiodan penetrates washed human erythrocytes slowly We find that hippuran and diodrast added to human and dog blood can be recovered from the plasma to the extent of 95 to 97 per cent within the first 30 minutes, as is the case with phenol red (8) This slight deficit we believe to be owing to incomplete centrifugation during the determination of the hematocrit, and we conclude that human red blood cells are for all practical purposes impermeable to these compounds In view of this fact it follows that all the phenol red hippuran, or diodrast excreted by the kidneys is carried to these organs by the plasma, and it is only necessary to divide the plasma clearance by the per cent of plasma in whole blood to obtain the corresponding whole blood clearance.

The question of how closely the diodrast clearance approaches a complete clearance can be answered only by determining what percentage of diodrast is removed from the renal blood, a measurement that requires the simultaneous analysis of blood from the renal vein and the systemic circulation Observations of this nature have been made on subjects with presumably normal kidneys and will be reported elsewhere (16) For all practical purposes it appears that the diodrast clearance approaches closely enough a complete clearance to be considered identical with the renal plasma flow

b Relation of plasma concentration to self-clearance The phenol red clearance begins to be significantly depressed when the plasma concentration of phenol red is elevated above 1 0 mgm per cent (8) and, except in the observations in Figure 1 where the concentration was raised to a

high level, our clearances have all been determined within the range of 0 7 to 1 0 mgm per cent

Numerous observations of a similar nature to those shown in Figures 2 and 3, and which we omit from this record for the sake of brevity, indicate that the diodrast and hippuran clearances are not appreciably depressed at plasma concentrations below 5 mgm per cent of iodine The lowest concentration at which great accuracy for the analytical method can be claimed, using 5 cc. of plasma, is 0 5 mgm per cent of iodine, hence there is ample latitude in the plasma concentration at which the clearances of these substances can be examined

c Simultaneous excretion of phenol red and iodine compounds When the plasma level of phenol red is elevated, the simultaneous diodrast clearance is depressed, even though the concentration of diodrast in the plasma is maintained below the level where self-depression begins (See circles in Figure 1, where the diodrast clearance was followed at elevated plasma levels of phenol red) Similarly, elevation of the plasma level of diodrast or hippuran depresses the simultaneous phenol red clearance, as shown by the circles in Figures 2 and 3 Diodrast and hippuran have a powerful effect in depressing the phenol red clearance, whereas phenol red has only a moderate effect in depressing the diodrast clearance (The action of phenol red on the hippuran clearance has not been examined) Essentially the same depression in the phenol red clearance is obtained when the concentration of diodrast or hippuran is allowed to fall from high levels as when it is rising from low levels The depression of the phenol red clearance by diodrast is not a transient one, when the concentration of diodrast is main-

creted by a common tubular mechanism that they enter into quantitative competition for this mechanism, and that diodrast and hippuran have a relatively great, and phenol red a relatively small, affinity for this mechanism No other substances are known which specifically affect these clearances, and it is probable that there is no substance normally present in plasma in concentrations sufficient to have an appreciable effect of this nature

Diodrast and hippuran displace phenol red from its combination with plasma protein and thus increase the free and filtrable fraction (17) When the plasma concentration of either iodine compound is raised to a sufficient extent the phenol red clearance is depressed below the inulin clearance, and nearly down to the level to be expected on the basis of the filtration of free phenol red (Figures 2 and 3) This confirms the thesis that it is only the free dye in the plasma that is available for filtration, and that the true filtration clearance of dye is given by the product of the inulin clearance and the fraction of free dye

For several reasons it is desirable to observe the phenol red and iodine clearances simultaneously in certain experiments pertaining to renal blood flow, as well as in the examination of the diseased kidney It is therefore necessary to know the critical level at which the iodine compounds first significantly depress the phenol red clearance Data on this point are given in Figure 4 In these observations the control phenol red and inulin clearances were determined in three periods of about 10 minutes each, a small quantity of iodine compound (3 to 10 cc of solution) was then injected into the infusion tubing some distance from the needle, the injection being made slowly (1 cc. per minute) [2]
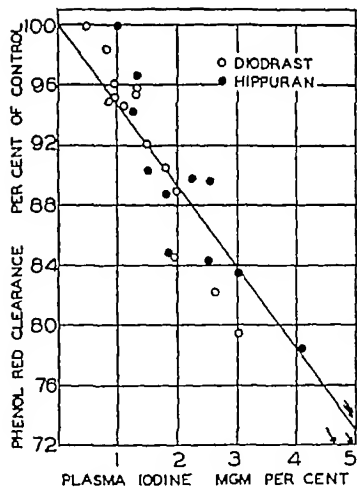
FIG 4 THE EFFECT OF SMALL CONCENTRATIONS OF DIODRAST OR HIPPURAN (EXPRESSED AS IODINE) UPON THE PHENOL RED CLEARANCE, AS REVEALED BY CHANGES IN THE ABSOLUTE VALUE OF THIS CLEARANCE OR IN THE PHENOL RED/INULIN CLEARANCE RATIO

In drawing the solid curve, consideration is given to the depression caused by higher concentrations of the iodine compounds as noted in such observations as those recorded in Figures 2 3 and 5

From a number of observations we have selected those in which renal circulatory disturbance is apparently minimal, and conclude that at 1 0 mgm per cent of iodine, neither diodrast nor hippuran depress the phenol red clearance by more than 5 per cent. Conversely, at 1 mgm. per cent (see Figure 1) phenol red does not appreciably depress the diodrast clearance, so that simultaneous clearances may be accepted as maximal for both substances in the normal kidney at plasma concentrations below these respective levels It would appear proper, where these clearances are determined simultaneously to make a correction on the phenol red clearance such as is indicated by the data of Figure 4

d On the possibility of storage of phenol red etc in the kidney It would seem that the most satisfactory method of examining the possibility of storage in the renal parenchyma would be to observe the behavior of the tubules in respect to excretion rate at a time when the plasma concen-

tration was rising and had never been higher than at the moment of observation, then, to raise the plasma concentration to higher levels, thus enabling the tubules to take up and store the solute if there is any tendency for them to do so, and then, allowing the plasma level to fall as rapidly as possible, to examine the behavior of the tubules at the reduced plasma level again, at a time when the stored material might be excreted in excess of current delivery by the blood Goldring, Clarke, and Smith (8) have reported observations of this type on the excretion of phenol red in man which show that this clearance has essentially the same value on both rising and falling curves

Since the publication of these experiments Elsom, Bott, and Wälker (5) have observed in the rabbit that the clearance of hippuran and more particularly of phenol red may considerably exceed the renal blood flow as measured by the thermostromuhr method, and they have concluded that these substances are stored in the kidney This point is of such fundamental importance, both in the measurement of the tubular excretory mass and the effective renal blood flow, that we have carefully examined the excretion of both hippuran and diodrast in man for evidences of storage by the method outlined above However, instead of using the hippuran and diodrast clearances themselves as the critical indicator of storage, we have used the depression of the phenol red clearance, the reason for this choice being that the depression of the phenol red clearance is a much more sensitive indicator of the presence of hippuran or diodrast in the plasma, and therefore in the tubule cells, than are the self-clearances, a very small increment in the concentration of the iodine compounds, insufficient to produce appreciable depression of the self-clearance, produces a marked depression of the phenol red clearance The details of these experiments are given in the protocols, but the general plan may be outlined here The plasma concentrations of inulin and phenol red were kept constant by suitable intravenous infusions while (*a*) hippuran or diodrast was introduced into the circulation in increasing concentration and raised to a high level, the administration of the iodine compound was then stopped and (*b*) the plasma level was allowed to fall, the rate of fall being extremely rapid since the clearances are large. Clearances obtained in
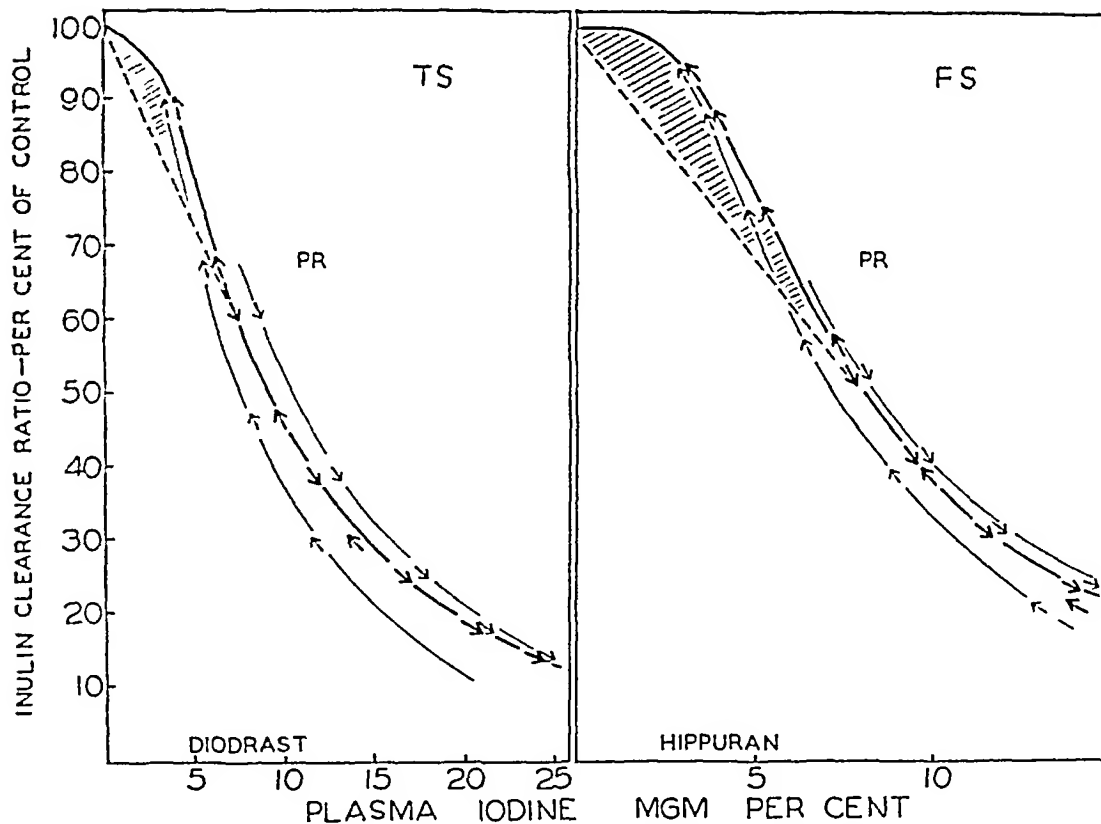
FIG 5 (LEFT) AND FIG 6 (RIGHT)   OBSERVATIONS DESIGNED TO EXAMINE THE POSSIBILITY OF STORAGE OF DIODRAST OR HIPPURAN IN THE RENAL TUBULES

The light arrows indicate the effect of diodrast (left) and hippuran (right) on the phenol red/inulin clearance ratio when the plasma concentration of the iodine compound is rising, and again when it is falling rapidly   The heavy arrows indicate these clearances corrected for a 150 second delay time   The identity of the "rising" and "falling" curves when so corrected indicates that the tubule cells return to the initial equilibrium, as revealed by the urine they elaborate, very quickly after that initial equilibrium has been disturbed by exposure to high concentrations of diodrast or hippuran.

Observations such as those in Figure 4 indicate that the phenol red clearance is depressed in an almost linear manner at the lower concentrations of the iodine compounds, the elevation of the phenol red clearance above the expected level, as shown in the shaded areas, is attributed to the accumulation of phenol red in the interstitial fluid of the kidney   This accumulation is, however, a negligible fraction of the total phenol red which passes through the kidney during the time the phenol red clearance is depressed.

this manner are recorded by the light arrows in Figures 5 and 6   During (b) the phenol red/inulin clearance ratio does not retrace the course so during (c), but is displaced first below and subsequently above the expected course

Analyzing this result it must be recognized that a particular sample of urine is formed from blood, not of the average composition possessed by the small venous systemic venous blood[3] but of the

average composition existing some seconds before the urine collection period   This interval, which we may designate the "total delay time," is made up of (1) circulation time from antecubital vein to right heart to renal artery to capillary plexus around the tubules, (2) diffusion time from capillary stream through interstitial fluid, (3) penetration into tubule cells, reaction with the excre-

method of interpolation is just as satisfactory as that recommended by Winkler and Parra (21) when as is the case with these observations, the change in P is exponentially related to time.

tory mechanism and passage across cells to tubule lumen, (4) dead space time, *i e,* the time required for the urine to pass down the tubules, through the pelvis and ureters, to enter the bladder By the specified conditions of its measurement, the total delay time must include any interval elapsing before the activity of the tubules is readjusted to a previously existing equilibrium after this equilibrium has been seriously disturbed, therefore, "storage" will manifest itself by an increase in interval 3

The divergence between the rising and falling curves shown in Figures 5 and 6 constitutes a measure of the total delay time as defined above. In order to determine the magnitude of this total delay time it is only necessary to determine what correction must be applied to the blood curves in our experiments in order to superimpose the two sets of data Empirically this correction is found in Subject T S (diodrast) to be approximately 3 minutes, in Subject F S (hippuran), 2 minutes But a large fraction of this total delay time is owing to the factors enumerated above other than 3, these other factors we will designate as the "minimal excretion time," which may be estimated independently by determining the minimal time required for phenol red, when injected intravenously, to appear in the urine, the latter *being collected at short intervals by syringe and* catheter This observation was repeated 18 times on 10 subjects, the average minimal excretion time varying from 120 seconds at a urine flow of 20 cc per minute to 200 seconds at a urine flow of 1 cc. per minute At urine flows of 6 to 2 cc per minute, the minimal excretion time averaged 150 seconds This interval includes all the elements making up the total delay time except the time specifically required for the tubules to *arrive at an equilibrium rate of excretion in relation to a given plasma concentration,* if delay resulting from storage is appreciable it should be evidenced by a difference between the minimal excretion time and the total delay time. Whether such a difference exists can be tested by correcting the data in the figures by the average minimal excretion time of 150 seconds When so corrected, as shown by the heavy arrows, the phenol red clearances on the falling plasma iodine curve are identical with those on the rising curve, as indicated by the shaded area, except at the low plasma

levels of iodine. This latter phenomenon, we believe, is owing to the fact that during the time when the excretory capacity of the tubules for phenol red is greatly depressed by the presence of large quantities of diodrast and hippuran, free phenol red diffuses out of the capillaries and tends to accumulate in the interstitial fluid of the kidneys in a concentration greater than that present when excretion is proceeding normally, on liberation of the excretory mechanism by removal of the iodine compound, this excess concentration of phenol red is available for excretion and, as calculated on the systemic plasma concentration, the clearance rises to slightly supernormal values One may speak of this phenomenon as "storage" of phenol red in the kidney as a whole, but not in the renal tubules, and it is negligible in magnitude even under the conditions of these observations Although the phenol red clearance was depressed below 20 per cent of its normal value, the fraction of phenol red accumulating in the kidney was less than 2 per cent of the total quantity which would have been excreted had the clearance remained at its normal level In no sense can the phenomenon be interpreted as indicating storage of diodrast or hippuran, which would depress rather than elevate the phenol red clearance.

The above observations show that about 2 to 3 minutes are required for the renal tubules to return to the initial equilibrium, as indicated by the phenol red clearance, after the plasma concentration of hippuran or diodrast has been elevated and allowed to fall again This interval is not appreciably greater than can be explained by physiological processes (1, 2, and 4 enumerated above) other than the time required for the re-equilibration of the excretory mechanism itself, and consequently it appears that the latter process is extremely rapid It is concluded that hippuran and diodrast are not stored to a significant extent *in the tubules during the process of excretion* from low or moderate plasma levels, and in view of Goldring, Clarke and Smith's (8) observations we may extend this conclusion to phenol red. This conclusion is fortified by the observation that phenol red is transferred from the peritubular fluid to the tubular lumen in a diffuse state, *i e,* without accumulation in the vacuoles or granules of the tubule cell (2)

It would seem appropriate, wherever either glomerular or tubular clearances are determined on a rapidly rising or falling plasma curve, to introduce a correction for delay time by interpolating the blood curve, not to the middle of the urine collection period, but to a point earlier by an appropriate interval Subsequent mention of the fact that data have been corrected for delay time means that we have deducted 150 seconds

from the nominal middle of the urine collection period in this manner

The exclusion of the possibility of storage enables us to approach the problem of measuring the tubular excretory mass and the effective renal blood flow with greater confidence In the measurement of the latter, as indeed in all clearance determinations, it is desirable to maintain the plasma concentration of diodrast or phenol
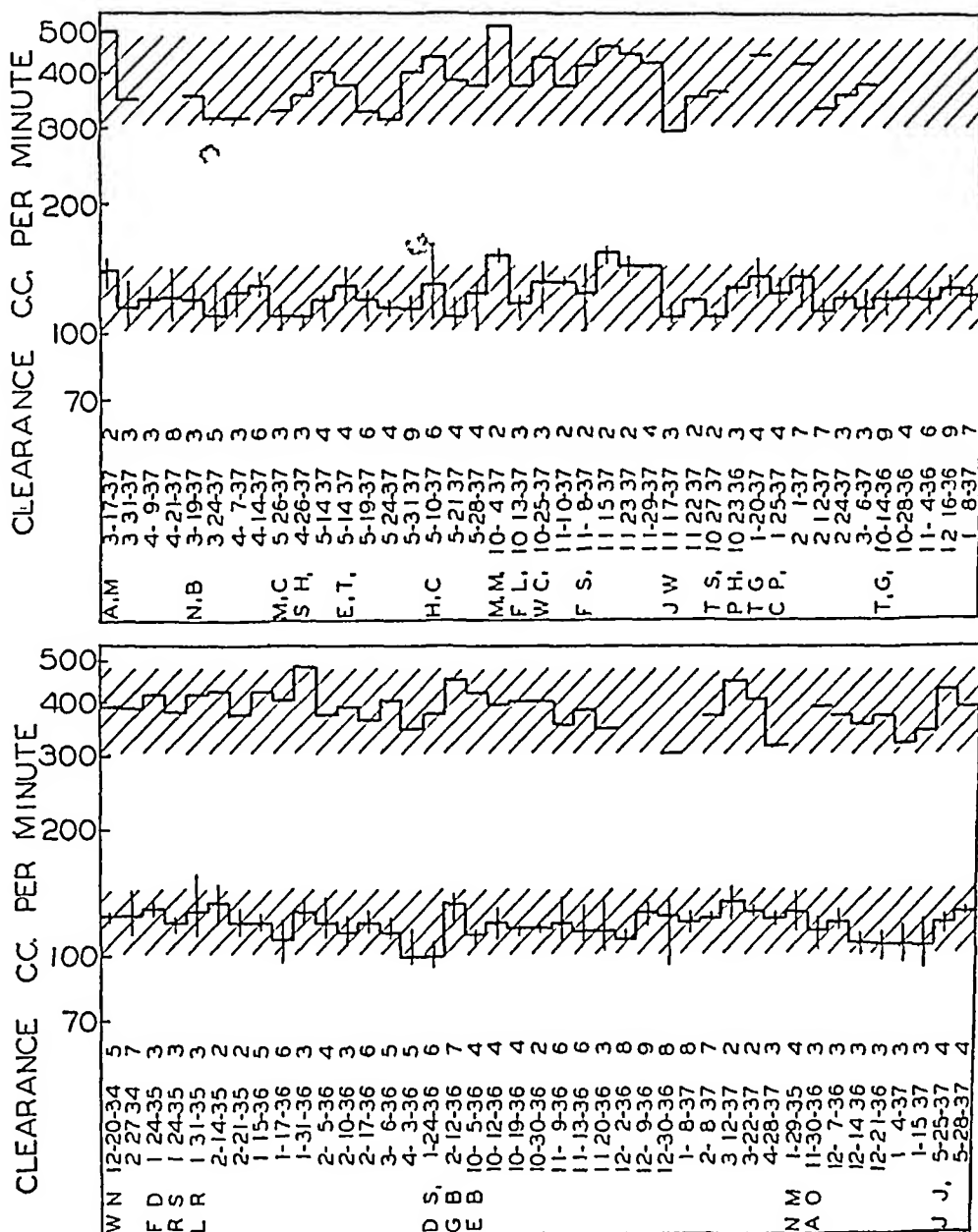
Figs 7a AND b   Inulin (Lower) and Phenol Red (Upper) Clearances in Normal Subjects, Corrected to 1 73 sq m Surface Area

red at a constant level by continuous intravenous infusions, so that no correction need be made for delay time. Knowing that the tubule cells re-equilibrate themselves within 150 seconds when the plasma concentration is changing, we believe that re-equilibration will occur within the same interval where there are marked changes in the rate of renal blood flow, a circumstance which might be encountered when the action of drugs, etc., is being studied.

Inulin and phenol red clearances in normal man

A limited series of observations on the inulin and phenol red clearances in normal man have been given by Shannon and Smith (13) and Goldring, Clarke, and Smith (8) Our present data include 25 volunteer, afebrile convalescent patients who showed no immediate evidence or history of circulatory or renal disease. Attention is especially called to the methods of preparing and administering the intravenous infusions, the collection of urine, *etc*, described under Methods The concentrations of phenol red and inulin in the plasma were generally maintained at constant levels ranging, respectively, from 0 7 to 1 0 and 100 to 150 mgm per cent Since these clearances are essentially independent of urine flow this datum has been omitted from the summary Lack of space prevents the presentation of the data in detail, but the pertinent features are given in Figures 7a and b and in Table III

The uppermost column of data in the figures *gives the number of clearance periods and the* vertical lines indicate the extreme high and low values of the inulin clearance, in each group of observations The average inulin clearance, crediting each subject once only, is 122 5 $\sigma = 10$ 7 The shaded bands indicate a range of 2 times the standard deviation Physiologically it would appear that values falling within $2 \times \sigma$ are normal, between $2 \times \sigma$ and $3 \times \sigma$ suspect and outside $3 \times \sigma$ definitely abnormal

Both the inulin and phenol red clearances in various individuals are quite constant as is demonstrated by the fact that the standard deviations of the average inulin and phenol red clearances are only 8.7 and 11 4 per cent of the respective means Except in four instances (Subjects D S, N B, M C and J W) the correlation between the simultaneous phenol red and inulin

clearances is even closer Subject D S was a morphine addict who showed marked withdrawal symptoms on a subsequent examination, and it is possible that there was abnormal vasomotor activity in the kidneys on the single experiment reported here. Nothing known about Subjects J W, N B, or M C would indicate renal disease, and all four subjects have been included in the calculation of the mean ratio

Viewing the phenol red clearance as proportional to renal blood flow, the constancy of the relative values of the phenol red and inulin clearances indicates that the filtration fraction in the normal kidney is remarkably uniform It has been our experience that vasomotor disturbances

TABLE III

*Summary of urea, inulin and phenol red clearances in normal man *

Subject	Sur face	Number of occasions examined	Number of inulin periods	Aver age per cent of plasma	Plasma clearances			Phenol red/Inulin
					Urea	Inulin	Phenol red	
	sq m.			*per cent*	*cc. per 1.73 sq m. per minute*	*cc. per 1 73 sq m. per minute*	*cc. per 1 75 sq m. per minute*	
W N	170	2	12			134	353	3.13
F D	1.56	2	7			130	418	3.21
R S	177	1	3			119	376	3.17
L R	1.69	11	47	0.61	60.5	119	402	3.37
D S	1.55	1	6	0.52		100	378	3.78
G B	1.70	1	7	0.49	84.8	133	458	3 44
E B §	1.73	15	77	0.58	67.0	133	353	3.14
A Q	1.80	6	18	0.59	65.0	111	354	3.25
T Q ‖	1.85	7	37	0.56	67.2	126	445	3.25
C P	1.91	5	20	0.58	63.6	122	398	3.25
A M ¶	1.69	4	16	0.58	70.8	134	419	3.33
N B **	1.78	4	17	0.57	72.6	121	327	2.70
M C	1.73	1	3	0.55		106	325	3.01
J J	1.73	2	8	0.63		125	413	3.50
S H	1.74	2	7	0.61		118	377	3.33
E T	1.90	4	23	0.56		110	355	2.98
H C	1.70	3	14	0.50		131	400	3.30
M M	1.75	1	3	0.63		153	518	3.39
F L	1.51	1	3	0.50		117	375	3.20
W C	2.05	2	5	0.56		133	409	3 10
F S	1.54	4	10	0.55		141	441	3.13
J W	1.69	2	5	0.60		115	325	2.83
T S	176	1	2	0.55		110	370	3.36
N M	1.47	1	4	0.55	82.0	129		
P H	1.75	1	3	0.56	73.0	123		
Mean					70.7 ±7.4	122.5 ±10.7	394 ±45	3.23 ±.25

Omitted from average although lacking definite history of renal disease.

| O B | 1.74 | 7 | 30 | 0.60 | 58.0 | 96 | 279 | 3.21 |
| J C | 1 64 | 5 | 15 | 0.59 | | 93 | 289 | 2.89 |

* For general methods of determination see Methods.
† Urine flow above 2 0 cc. per minute
‡ Based only on periods when both inulin and phenol red clearances were determined simultaneously
§ Phenol red clearance absent in 17 periods.
‖ Phenol red clearance absent in 33 periods
¶ Phenol red clearance absent in 11 periods
** Phenol red clearance absent in 6 periods

(febrile reactions, drugs, *etc*,) are immediately reflected by changes in the phenol red/inulin clearance ratio, in general the inulin clearance tending to fall, the phenol red clearance to rise, a divergence which can be explained by the supposition that the normal glomerular pressure is maintained by tonic constriction of the efferent glomerular arteriole Dilatation of this vessel, in this view, would lead to a decrease in the filtration fraction with perhaps a simultaneous increase in renal blood flow

The inulin clearance in the same subject on various occasions is also quite constant Subject E B, for example, was examined 15 times in a period of a year and showed an extreme range of the average inulin clearance from 113 to 137 cc per minute with a mean clearance of 122 cc

The relative constancy of the inulin clearance and the phenol red/inulin clearance ratio in the present series is perhaps attributable in part to the fact that the observations have been made under standardized and fairly basal conditions the subjects were examined in the morning without breakfast and with a minimum amount of physical activity, to produce a uniform degree of hydration, one to two liters of water are administered the day previous and two liters on the morning of examination (from 5 30 to 8 30 a m) the last water being taken at least 90 minutes before the first clearance period But contributing equally to this constancy is the fact that the technique of clearance determination is designed to give the most accurate results obtainable The use of constant, slow infusions gives reliable blood curves of inulin and phenol red, and catheterization of the bladder followed by a careful washing with saline eliminates what is perhaps the largest source of error This last precaution is obviously necessary if single clearance determinations are to be given any physiological significance It is at times difficult to obtain complete emptying of the bladder even when a multiple-opening catheter is used and the bladder is washed out with 20 cc of saline

It is appropriate to note in this connection the variability to be expected in single, successive clearance determinations We have subjected our individual inulin clearance determinations to analysis as follows the extreme high and low values of this clearance in each series of observa-

tions (as shown by the vertical lines in Figu 7a and b) have been used to calculate a standa deviation relative to the average value of all servations on that day taken as unity The stan ard deviation thus calculated for 356 periods ± 0 089, 69 5 per cent of the determinatic falling within once, 96 per cent within twice a 100 per cent within 3 times the standard de ation It follows that a variation of any individ clearance, from the average of a series of cle ances on one day, of more than 8 9 per c should not occur oftener than 30 5 times, and variation of more than 17 8 per cent should occur oftener than 4 times, in 100 determinatio Unexpected changes in renal activity do unqu tionably occur in a subject on repeated examir tion, but in no instance are these changes of su a magnitude as to invalidate the physiological s nificance of the average of a series of three four observations on any one day

We conclude from the present data on norm subjects that renal activity, particularly as i vealed by the inulin clearance, is remarkably cc stant under standard conditions, not only in t same subject in successive 15 to 20 minute perio but week by week, this clearance is also relative constant in different subjects We believe th the highly variable clearances reported by oth investigators are attributable in great part various technical errors, and chiefly to failure empty the bladder completely

In considering whether or not the data in Tal III furnish reliable standards for normal ren activity, attention should be called to the fact th the two individuals with low inulin clearanc shown at the bottom of the table have been e cluded from the calculation of the average figur Though nothing in the history of these individua indicates renal disease, it was felt that this cou not confidently be excluded

We have included the urea clearance in sor of our observations because of the great practic importance of this determination Contrary the usual practice, we determine urea concentr tion in the plasma rather than in whole blood, practice defensible on physiological grounds sin the calculation of the fraction of urea reabsorb by the tubules is based upon the plasma rath than the whole blood clearance If allowance made for the difference between plasma an

whole blood clearance, our average figure is in agreement with the widely accepted figures of Möller, McIntosh, and Van Slyke (11)

SUMMARY

The data given in Table II are too limited in number to establish normal values, but so far as the six individuals reported there are concerned, the minimal renal plasma flow, as measured by the diodrast clearance, has an average value of 820 cc per 1 73 sq m surface area per minute. This corresponds to a minimal whole blood flow of 1384 cc   Although there is little reason to believe that such is the case, these figures may be slightly elevated by a vasodilator action of diodrast and by circulatory acceleration associated with clearance determination   Of 820 cc of plasma, an average of 137 cc. per minute are filtered, as indicated by the inulin clearance   Thus the average filtration fraction is 16 7 per cent. Applying this figure to the average inulin clearance, as given in Table III, it appears that the minimal, basal effective blood flow through the two kidneys of ideal man averages about 1300 cc. per minute

Observations on the extent to which the tubular excretory mass and the effective renal blood flow may be altered by various physiological or pharmacological agents, or in the course of disease, are now in progress and will be reported at a subsequent time, with supplementary data on the normal value of T_m.

CONCLUSIONS

1a. Methods are described for measuring the tubular excretory mass and the effective renal blood flow in the human kidney   These methods are based on the capacity of the renal tubules to remove certain foreign substances from the blood and to excrete them into the urine independently of the activity of the glomeruli

b Data on a limited series of subjects are given for the effective renal blood flow and the fraction of plasma filtered at the glomerulus

2 As subsidiary matters of physiological importance in the development of these methods, it is shown

a That phenol red, diodrast, and hippuran are excreted by a common cellular mechanism in the tubules

b That tubular excretory activity is limited, in that there is a maximal rate of excretion for each substance

c That no significant quantity of diodrast or hippuran (or, from previous data, of phenol red) is stored in the renal tubules

3 Values are given for the inulin and phenol red clearances in normal man   These clearances are shown to be quite constant from time to time in the same subject and in different subjects

METHODS

1 *Preparation of infusion fluid*   To establish and maintain a blood plateau in the shortest time a priming infusion (designated here as Number 1) is given at 10 cc. per minute, followed by one or more sustaining infusions of appropriate composition at the rate of 4 cc. per minute.

The preparation of *inulin* suitable for intravenous administration has been described elsewhere (15), impure preparations may produce severe reactions and it is imperative that a physiologically certified preparation be used   We have recently removed the pyrogenic agent by filtering the inulin solution before use through a Seitz E.K. asbestos filter, as recommended by Co Tui et al (3)   Since inulin has a very high molecular weight (20) it has negligible osmotic properties and it must be administered in saline (0.9 to 1 0 per cent)   The solution is prepared by dissolving the inulin in sterile saline with the aid of heat and filtering while hot through a Seitz filter if the inulin is pyrogenically reactive, it is then boiled for 5 minutes in a loosely stoppered flask to effect sterilization, and sterile phenol red, hippuran etc are added.   The infusion flask and tubing are filled with hot saline to remove air, this is then drained off to the bottom of the infusion flask, and after the inulin solution has been transferred to the flask a volume of fluid corresponding to the dead space of the infusion tubing is drained off in order to bring the inulin solution down to the needle.   Inulin is only slightly soluble in cold water, and although it readily forms a supersaturated solution when heated, the solution should be transferred to the infusion flask while hot to prevent crystallization.   A 10

per cent inulin solution will usually remain at body temperature for one hour without crystallization, while a 3 per cent solution keeps several hours It is our practice to pass the fluid through a glass coil immersed in a water bath at body temperature, since with this precaution the fluid in the infusion flask may be started at a considerably higher temperature A T-tube carrying a thermometer is inserted in the infusion tubing between the cooling coil and the needle, to afford a check on the temperature of the infusion fluid as it enters the arm Where it is desirable to change the infusion fluid during the course of a series of observations, this can be done quickly by withdrawing the adapter from the needle, draining the old infusion fluid to the bottom of the flask, substituting the second infusion fluid and discarding a volume of fluid equal to the dead space of the tubing A special tunnel clamp which gives uniform compression over the infusion tubing for a distance of 4 inches is used to control the rate of infusion, which is measured by means of a graduated pipette communicating with the infusion flask by a Y-tube The emptying time of this tube is noted with a stop watch

Where low urine flows may result from experimental procedures, diuresis should be maintained by incorporating 2 per cent Na_2SO_4 in the infusion fluid, to prevent inulin from crystallizing out in the urine.

2 *Collection of urine and blood* Urine is collected by an inlying catheter, the urine being allowed to drain into a narrow-necked flask during the collection period Toward the close of the period air is blown into the bladder by syringe and the last of the urine removed by suction, the bladder is then washed out with an accurately measured volume of saline and a small quantity of air The termination of the period is timed as closely as possible to the removal of the last of this wash fluid A sterile catheter is used and sterile gloves are worn The urine and wash fluid are combined for analysis, and a preliminary 1 10 dilution is made at once to prevent precipitation of the inulin

Blood samples are drawn from the antecubital vein, using double strength colorless heparin (Connaught Laboratories) to prevent clotting The blood is centrifuged at once, the plasma separated, and an accurately measured 2 cc. sample is set aside for inulin analysis Oxalate is added to the rest of the plasma which is used for phenol red and hippuran analysis Two cc. of plasma are required for inulin, 2 cc. for phenol red and in general 10 cc. for duplicate iodine analysis

3 *Analytical methods* Inulin analyses are carried out on copper sulphate-sodium tungstate filtrates (18), the plasma and urines being diluted 1 8 or 1 10 in precipitation, the urines first being diluted to the approximate U/P ratio as calculated from the urine flow and probable inulin clearance. Glucose is absorbed from the filtrates of both plasma and urine by treating 5 cc of filtrate with 1 cc. of packed yeast which has previously been well washed to remove all reducing substances The filtrate is left in contact with the yeast for 15 minutes, with occasional stirring, before being centrifuged again

The method of inulin analysis now in use and which has been repeatedly tested for recoveries of inulin is as follows

Two tenths cc. N H_2SO_4, accurately measured from a capillary pipette, are carefully placed in the *bottom* of a Folin sugar tube. Two cc. of filtrate are introduced on top of the acid, delivering this filtrate against the constriction of the tube The mixture is agitated slightly and the inulin is hydrolyzed by placing it in boiling water for 15 minutes Two-tenths cc. of N KOH are then pipetted against the constriction and a drop of phenolphthalein is added If the mixture is alkaline a drop of 0 1 N H_2SO_4 is added When acid, 1 per cent Na_2CO_3 is added drop by drop until the mixture is a permanent pink This should not require more than 2 drops of Na_2CO_3, and the use of a larger quantity introduces perceptible error in the glucose determination Two cc. of the copper tartrate solution (6) are then added and the inulin is determined as fructose. The simultaneous bloods and urines are boiled together and read against a common standard All sugars are read in a Duboscq colorimeter against a single standard containing 15 mgm per cent of glucose which has been boiled with the unknowns Inulin recoveries from both blood and urine have been satisfactory from 7 5 to 25 mgm per cent in the filtrate (It should be noted that the use of a single standard as described above does not give complete recoveries of glucose throughout so wide a range.) By this method inulin has a glucose equivalent of about 100 per cent, and all figures are reported as apparent glucose. Six bloods and fifteen urines can be analyzed in duplicate in this manner in an afternoon and morning

Prior to *phenol red* analysis the oxalated plasma is centrifuged at high speed in heavy walled Pyrex tubes to remove oxalates and red cells, hemolysis of the latter introducing error Two cc of plasma are alkalinized with a drop of saturated Na_2CO_3 and compared with 2 cc of a 1 0 mgm per cent phenol red solution similarly alkalinized in a Duboscq microcolorimeter If, on alkalinization, the plasma becomes cloudy it is necessary to centrifuge again Reading should be done within 30 minutes An ϵ 74 Wratten filter is placed in the eyepiece and the colorimeter is illuminated by a Photoflood bulb with a variable resistance If it is necessary to dilute the plasma before reading in the colorimeter, saline must be used as a diluent to prevent precipitation of the globulins Analysis of the diluted urines is carried out in a similar manner

Where the plasma level of phenol red is kept close to 1 0 mgm per cent, a single blank determination is made by adding 1 0 cc of 2 5 mgm per cent phenol red solution to 2 0 cc of the patient's plasma drawn prior to the first infusion This is read against a 1 0 mgm per cent standard and the apparent blank determined by subtracting 0 83 and multiplying by 1 5 This blank (0 to 0 10 mgm per cent) is deducted from all plasma phenol red readings Each sample of plasma is tested for hemolysis by a slight modification of Bing and Baker's (1) hemoglobin method 2 cc. of a 2 per cent benzidine solution

in 20 per cent glacial acetic acid are added to 0 4 cc. of plasma plus 0 6 cc. of water the mixture agitated, and 1 0 cc. of 1.5 per cent H_2O_2 added. A blank using 1 0 cc. of water is prepared in the same manner The solutions are grossly compared after 1 hour, and if hemolysis is present the phenol red determination is discarded.

Iodine analysis is carried out by Kendall s (9) method, with slight modifications which may be briefly noted to supplement the original description All samples are dried at 90 C. before fusion with NaOH. Heat resistant glass beads (15 to 20) are used to prevent boiling over after use these are washed boiled with dilute H_3PO_4 washed and dried at 90° Only 2 cc. of 20 per cent sodium bisulphite are used instead of 5 cc. 1.5 cc. excess of H_3PO_4 are added after the methyl red end point is reached, and the final addition of reduced H_3PO_4 is omitted. Sodium thiosulphate is standardized by titration of 5 cc. of N 0 001 KIO_3 added to a dummy prepared of NaOH, H_3PO_4, *etc* as in the analysis of unknowns. All reagents are carefully examined for a blank. Thirty five analyses of KIO_3 or KI varying from 25 γ to 100 γ added to plasma have given recovery with a S.D of $\pm 1 44 \gamma$ Similarly diodrast added to plasma in quantities less than 100 γ has been recovered with a S D $\pm 1 84 \gamma$ (20 observations) Seventy per cent of routine determinations check within 2 per cent 93 per cent check within 5 per cent.

Ureas are determined by Van Slyke s (19) manometric urease method, using plasma and diluted urines, digestion in each case being carried out in the burette.

Plasma proteins are determined by Wu s (22) method with the addition of lithium sulphate to the phenol reagent (7)

4 *Protocols* Since many of the observations recorded here involve infusion fluids of special composition the more important features of these fluids and their manipulation will be reviewed. For brevity the following abbreviations are used phenol red, PR. diodrast D, hippuran, H urine collection period, U blood sample, B The time at the beginning of the priming infusion is indicated by 0 and the elapsed time thereafter by 10' 15, etc. Phenol red is used as a sterile 10 per cent solution prepared for us by Hynson, Wescott and Dunning diodrast is the 35 per cent sterile solution marketed by the Winthrop Chemical Co., and hippuran is the 48 per cent sterile solution marketed by the Mallinckrodt Chemical Company U_1 begins about 20 minutes after infusion No 2 is started.

Figure 1 Inf No 1 15 gm inulin 1.5 cc. PR 1 cc. D in 100 cc. saline from 0 to 10 Inf No 2 50 gm inulin 10 cc. PR. 10 cc. D in 1000 cc. saline at 4 cc. per min until end. From 60 to 67.5 70 cc. 10 per cent PR. solution injected into infusion tubing near arm. Discard period from 59 to 73 Total time, 200', 14 urines and 7 bloods

Figure 2 Inf No 1 15 gm. inulin, 1.5 cc. PR., 1.5 cc. D in 100 cc. saline from 0 to 11 Inf No 2 20 gm. inulin 4 cc. PR., 4 cc. D in 400 cc. saline at 4 cc. per min. from 11' to 75' Inf No 3 25 gm. inulin, 2 cc.

PR., 25 cc. D in 500 cc. saline from 79' to end. From 84' to 89' 50 cc. D injected into infusion tubing Discard from 74' to 99' Total time, 186 11 urines and 6 bloods B_o to B_s at 0 40', 75', 98', 120' 150' and 186, respectively

Figure 3 Inf No 1 12 gm. inulin, 2 cc. PR., 1 cc. H. in 100 cc. saline from 0 to 11' Inf No 2 20 gm inulin 4 cc. PR., 4 cc. H in 400 cc. saline at 4 cc. per min. from 11' to 71' Inf No 3 30 gm. inulin, 2 cc. PR., 50 cc. H in 500 cc. saline from 72' to end. At 82' 91 5' and 102' 24 12 and 8 cc. H mixed with infusion fluid in flask. Total time, 197' 11 urines and 6 bloods.

Figure 4 Inf No 1 15 gm. inulin 1.5 cc. PR 1 cc. D or H in 100 cc. saline from 0 to 10 Inf No 2 30 gm. inulin 4 8 cc. PR., 6 cc. D or H in 600 cc. saline at 4 cc. per min. from 10' to end. After three control urine periods, from 3 to 10 cc. of D or H were injected slowly into infusion tubing or directly in vein of other arm. Total time, about 120, 8 urines and 5 bloods

Figure 5 Inf No 1 12 gm. inulin and 1.5 cc. PR. in 100 cc. saline from 0 to 10' Inf No 2 12 gm inulin, 2 25 cc. PR. 300 cc. saline, 10 to 52' Inf No 3 16 gm. inulin, 2.0 cc. PR., 40 cc. D in 400 cc. saline, 53.5 to 120 5' At 59.5 and 70 respectively 12 and 6 cc. D added to infusion flask. Inf No 4 12 gm inulin and 2.25 cc. PR. in 300 cc. saline from 120.5 to end. Discard periods from 52' to 64 and 118.5 to 130' Total time, 159' 11 urines and 7 bloods

Figure 6 Inf No 1 15 gm. inulin and 1.5 cc. PR. in 100 cc. saline from 0 to 10 Inf No. 2 15 gm. inulin and 2.25 cc. PR. in 300 cc. saline from 10' to 50 Inf No 3 20 gm. inulin, 1 6 cc. PR. and 47 cc. H in 400 cc. saline 52' to 115 5 At 57' and 68 respectively 13 and 7 cc. of H added to infusion flask. Inf No 4 15 gm. inulin and 2 0 cc. PR. in 300 cc. saline, 115.5 to end. Discard period 62' to 73 Total time, 157' 13 urines and 8 bloods

Tables I II and III The routine examination of normal individuals of 17 sq m. S.A. may be accomplished as follows Inf No 1 15 gm. inulin, 1 cc. PR. and 1 cc. D in 100 cc. saline from 0 to 10' Inf No 2 15 gm inulin, 3 cc. PR. and 3 cc. D in 300 cc. saline 10 to end at 4 cc. per minute. These infusions will give 100 to 150 mgm per cent of inulin, 0.75 to 1 0 mgm. per cent of PR. and 0.5 to 1 0 mgm. per cent of D in the plasma, depending on clearances. B_o for PR. blank may be drawn when starting inf No. 1 B_1 at 28', B_2 at 60' and B_3 at 92', and the bladder emptied and washed at 30' with U and U_1 at 45 U_2 at 60 U_3 at 75 and U_4 at 90 It was by essentially this technique that most of the data in Table III were collected, though a few of the earlier observations were made on falling blood curves after single intravenous injections. Where the clearance of any substance is reduced the concentrations in the infusion fluid should be reduced approximately in proportion to the square root of the per cent of normal clearance keeping the infusion rate at 4 cc. per minute.

Protocol 2 may be followed for measuring both maximal clearances and T_m

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THE EFFECT OF ALCOHOL ON THE WATER AND ELECTROLYTE BALANCE IN MAN

By WILLIAM M NICHOLSON and HAYWOOD M TAYLOR

(From the Departments of Medicine and Biochemistry Duke University School of Medicine, Durham)

The work presented here was undertaken primarily for the purpose of ascertaining the effect of alcohol in moderate quantities on the water and salt balance in man   It is a commonly observed fact that alcohol in man produces a diuresis and MacNider and Donnelly (1) have shown this to be the case in dogs   It is likewise well known that thirst is a prominent symptom during the recovery phase of acute alcoholism   No balance studies have been made of this condition, and it was thought that data could be collected which would explain some of the features observed

Alterations in the acid-base balance after the ingestion of alcohol was first shown by Thomas (2) in 1898, who reported that the carbon dioxide content and carbon dioxide capacity of the blood was diminished   Himwich and coworkers (3) in 1933, made observations on the acid-base balance in dogs and in man after feeding alcohol   Their studies were made over short periods of time, and no attempt was made to study the electrolyte balance, however, they observed no change in the total base or chlorides of the serum   They found that there was a reduction in the carbon dioxide content and carbon dioxide capacity of the blood which was accompanied by a fall in the blood pH There was also an increase in the blood lactic acid and sugar   It was their belief that the increase in lactic acid was brought about by the conversion of muscle glycogen to lactic acid by the action of alcohol   Futer and coworkers (4) in short experiments on dogs noted a fall in the carbon dioxide capacity, an increase in the blood sugar and an increase in the blood potassium content Gojcher and coworkers (5) reported the same findings in chronic alcoholism in man but made no attempt to study the excretion of the electrolytes

Wakai (6) using rabbits, was able to show that the serum protein concentration was decreased and was accompanied by a decrease in the serum and blood viscosity after the feeding of alcohol   Levin (7) has shown that in man the ingestion of alcohol caused an increase in blood volume in certain instances

EXPERIMENTAL

Healthy young adult male volunteers were used in the experiments. They were allowed to carry on their usual routine as students   A constant diet, which was prepared by the same individual throughout each experiment, was given. Two duplicate samples of the diet were analyzed for the potassium, sodium, chloride, water, and nitrogen content. The time at which fluid was taken and the amount were constant for each day throughout the experiment. A weighed amount of sodium chloride was supplied to the subject for use on his food for the day and all food was consumed.

In order to determine the changes that might occur in periods shorter than 24 hours the day was divided into three 8-hour periods. The urine was collected for each of the 8-hour periods, toluene added as a preservative, and the specimens were kept in the ice box until used. The stools were collected for 24-hour periods. The blood samples were collected anaerobically heparin being used as the anticoagulant (8)   A control blood was obtained 8 hours before the beginning of alcohol intake. The subject was given alcohol in the form of whiskey, gin or ethyl alcohol over a period of 8 hours when a second blood sample was obtained   The third and fourth blood samples were obtained 8 and 32 hours respectively after the end of the alcohol consumption. The time at which fluid was taken remained the same in all experiments, alcohol being substituted for an equal quantity of water   No alcohol was allowed during the last hour of the period of alcohol intake, and the subject was kept at rest in bed   Vomiting did not occur in any of the experiments   In Experiments 1, 2, and 3 the subjects received alcohol in the form of whiskey diluted with ice and water   In Experiment 5 the alcohol was given in the form of distilled gin diluted with water and ice, while in the 4th experiment ethyl alcohol diluted with ice and water was drunk. In two experiments (1 and 3) a low sodium chloride intake diet was given, 4 grams per day in Experiment 1, and 1 gram per day in Experiment 3 and in Experiments 2 4 and 5 an adequate sodium chloride intake was allowed (7 grams per day)

The columns headed   balance" in Table IV represent the difference between the output and intake of the various substances the output in each instance representing the total as measured by analyses of the urine,

stools, and withdrawn blood The intake represents the total amounts in the water, food, and sodium chloride taken by mouth Periods labeled 1, 2, and 3 are averages for the corresponding periods for two days and constitute controls Period 4 represents the time of alcohol intake and during Periods 5 and 6, it was considered that the subject was still under the influence of alcohol The recovery phase begins with Period 7

The methods for chemical analysis are as follows Estimations of the sodium were made by the method of Butler and Tuthill (9) A trichloracetic acid filtrate of the plasma was used instead of wet or dry ashing The filtrate was prepared in the manner recommended by Kerr (10) The potassium was estimated by the method of Shohl and Bennett (11), after ashing the sample according to the technique of Strauss (12) The chlorides were determined by the open Carius method as modified by Eisenman (13) The carbon dioxide content was measured by the method of Van Slyke and Neill (13) The pH determinations were made colorimetrically by the Hastings and Sendroy technique (14) Lactic acid estimations were made according to von Fürth-Charnass as modified by Friedemann and Kendall (13) The blood alcohol was determined by the method of Friedemann and Klaas (15) The macro-Kjeldahl method was employed for the determination of the nitrogen, in the plasma, urine, and stools (13) Nonprotein nitrogen was determined according to Folin and Wu (13) and the blood sugar by Benedict's method (13) All determinations were made in duplicate, unusual results were checked in triplicate

RESULTS

Five complete balance studies were made, the results of which were consistent regardless of the

form in which alcohol was taken Data from such experiments (Tables I, II, and III) reveal that there was a retention of sodium, chloride, and potassium A small amount of water was lost during the 24-hour period, but this loss was more striking during the 8-hour period in which the alcohol was given Diuresis was produced and occurred always in the first 4 hours of the period, so that during the last 4 hours only small amounts of urine were voided, even though alcohol was taken during this time Although there was a diuresis of water, the sodium, chloride, and potassium ions were retained and their concentration in the urine was lowered Accompanying the water diuresis, which occurred only while the subject was taking alcohol, there was a loss of weight greater than could be accounted for by the loss of water in the urine alone

The retention of the sodium and the chloride was more marked when the intake of these ions was high, and in the two experiments in which the sodium chloride intake was inadequate there was a negative balance for these ions, which, however, was not as great as in the control period (Table IV) In all experiments, the potassium retention (Table V) was marked and apparently had no relationship to the amount of sodium or chloride retained

We have considered the recovery day as beginning 16 hours after the end of the period of

TABLE I

Effect of alcohol when adequate sodium chloride was given Experiment 5 †

Period	Weight	Water excre tion *	Sodium excre tion *	Chlo ride excre- tion *	Potas sium excre tion *	pH of blood	Blood non protein nitro gen	Blood sugar	Blood lactic acid	Blood alco hol	Plasma chlo ride	Plasma so dium	Plasma potas sium	Plasma CO₂ content	Plasma pro tein
8 hours each	kgm	cc	m.eq	m eq	m.eq		mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc	m.eq per liter	m eq per liter	m.eq per liter	m.eq per liter	grams per 100 cc
Control 1	71 4	1000	40 8	63 6	31 0	7 38	32	88	6 65	8 55	100 5	144 1	3 8	27 5	6 57
2		760	48 4	40 1	12 8										
3		1010	43 5	58 6	42 2										
Alcohol 4	70 5	800	38 3	43 0	9 1	7 34	31	91	13 23	147 0	100 1	144 1	3 5	25 5	6 18
5		160	24 8	21 4	3 3	7 34	31	86	12 88	121 4	104 0	145 3	5 5	25 5	6 48
6		50	10 1	4 9	4 6										
7	71 9	450	28 4	44 1	42 4	7 38	27		13 59	10 26	102 0	142 5	3 4	26 7	6 14
8		1010	65 6	33 2	33 4										
9		1300	57 8	72 1	41 3										

* The excretion is the total amount of substances in the urine, stool, and withdrawn blood
† Diet of 2400 calories, 2400 cc of water, and 7 grams of sodium chloride were given daily   180 cc of 95 per cent alcohol in the form of distilled gin were given in the fourth period Periods 1, 2, and 3 are average figures for the corresponding periods during the control days Blood samples were collected at the end of the periods

TABLE II

Effect of alcohol when adequate sodium chloride was given   Experiment 4 †

Period	Weight	Water excretion*	Sodium excretion*	Chloride excretion*	Potassium excretion*	pH of blood	Blood non protein nitrogen	Blood sugar	Blood lactic acid	Blood alcohol	Plasma chloride	Plasma sodium	Plasma potassium	Plasma CO₂ content	Plasma protein
8 hours each	kgm.	cc.	m.eq	m.eq	m.eq		mgm per 100 cc	mgm. per 100 cc.	mgm per 100 cc	mgm per 100 cc.	m.eq per liter	m.eq per liter	m.eq per liter	m.eq per liter	grams per 100 cc.
Control 1	66 6	1680	52 2	72.3	37 3	7 40	26	94	7,92	4 2	105	141 9	2 8	26 8	6 60
2		500	27 1	18 7	9 9										
3		820	52 9	58 8	36.3										
Alcohol 4	66 2	1990	28 6	38 1	2 0	7.37	29	97	12.24	196 6	105.3	144 0	4 2	25 4	6.38
5		465	26 4	29.5	15.9	7.37	23	80	12 24	59 8	107 4	146.9	5 0	26 4	6.52
6		645	40.3	40.3	1 4										
7	66 6	1400	39 4	58 6	26 9	7 40	28	92	7 56	8.5	101 8	140.2	3 0		6 60
8		250	31.8	24 1	22 1										
9		1220	82 8	94.3	55 4										
10	66 8	1135	51.3	57 0	27.3	7 40	29	96	8 59	8.5	98 7	142 0	3 0		6 55
11		740	31.5	24 4	10 7										
12		1210	62 7	91 1	75.3										

* The excretion is the total amount of substances in the urine stool, and withdrawn blood
† Diet of 2400 calories, 2400 cc of water and 7 grams of sodium chloride were given daily   200 cc. of 95 per cent alcohol diluted with ice and water were given in the fourth period   Periods 1 2 and 3 are average figures for the corresponding periods during the control days. Blood samples were collected at the end of the periods.   In this experiment the recovery phase was prolonged one day

TABLE III

Effect of alcohol when inadequate sodium chloride was given   Experiment 3 †

Period	Weight	Water excretion*	Sodium excretion*	Chloride excretion*	Potassium excretion*	pH of blood	Blood non protein nitrogen	Blood sugar	Blood lactic acid	Blood alcohol	Plasma chloride	Plasma sodium	Plasma potassium	Plasma CO₂ content	Plasma protein
8 hours each	kgm	cc.	m.eq	m.eq	m.eq		mgm per 100 cc.	mgm per 100 cc	mgm per 100 cc	mgm per 100 cc.	m.eq per liter	m.eq per liter	m.eq per liter	m.eq per liter	grams per 100 cc
Control 1	94 2	1730	13 8	24 3	50 1	7 38	26	98	9 2	35.39	103 2	142 9	3 8	27 1	6 75
2		290	22 7	19 8	8 8										
3		880	23 0	36 2	24 1										
Alcohol 4	93 3	2450	19 8	14 1	16 9	7 32	23	138	12.87	232.5	104 9	143 1	7 9	24 1	7 03
5		225	16 7	20 7	14 5	7.35	25	102	13.59	104.3	103 2	145 6	8.3	24 6	6 94
6		242	8 7	18 2	12 8										
7	94 7	570	5.5	14 8	29 0	7.38	25	105	7 92	40.3	103 2	139 0	3.5	26 5	6.57
8		355	6 7	11 0	4 1										
9		1240	6.2	15.5	30 7										

* The excretion is the total amount of substances in the urine stool and withdrawn blood
† Diet of 2400 calories 2400 cc. of water and 1 gram of sodium chloride were given daily   Periods 1 2 and 3 are average figures for the corresponding periods during the control days   Blood samples were taken at the end of the periods. 212 cc. of 95 per cent alcohol in the form of whiskey were given during period 4

alcohol intake, at which time the blood alcohol had fallen considerably   Actually, however, the recovery day does not begin at this time since water, sodium, potassium, and chloride were retained in large amounts for the first period of that day (Period 7, Tables I, II, and III)   Nevertheless, the balances for the complete day reveal that the recovery phase was characterized by urinary excretion of potassium sodium and chloride, with retention of water   Since more potassium was retained during the period of alcohol consumption the loss of this ion was greater than for the sodium and chloride   The negative balance first day of the recovery period was

TABLE IV

Balance data on Experiments 3, 4, and 5 *

Experiment	Day	Intake				Output				Balance			
		Potassium	Sodium	Chloride	Nitrogen	Potassium	Sodium	Chloride	Nitrogen	Potassium	Sodium	Chloride	Nitrogen
		m.eq	m.eq	m.eq	grams	m.eq	m.eq	m.eq	grams	m.eq	m.eq	m.eq	grams
3	1	85	33 8	54 6	14 65	83 0	59 5	80 3	13 73	+ 2 0	−25 7	−25 7	+0 92
	2	85	33 8	54 6	14 65	44 2	45 2	53 0	13 80	+40 0	−11 4	+ 1 6	+0 85
	3	85	33 8	54 6	14 65	63 8	18 4	40 2	15 28	+21 2	+15 4	+14 4	−0 63
4	1	85	135 7	156	14 65	83 5	132 2	149 8	14 75	+ 1 5	+ 3 5	+ 6 2	−0 10
	2	85	135 7	156	14 65	19 3	95 3	107 9	12 21	+65 7	+40 4	+48 1	+2 44
	3	85	135 7	156	14 65	104 4	154 0	177 0	12 65	−19 4	−18 3	−21 0	+2 00
	4	85	135 7	156	14 65	113 3	145 5	172 5	14 17	−28 3	− 9 8	−16 5	+2 20
5	1	85	135 7	156	14 65	86 0	132 7	162 4	15 51	− 1 0	+ 3 0	− 6 4	−0 86
	2	85	135 7	156	14 65	17 0	73 2	69 3	11 95	+68 0	+62.5	+86 7	+2 70
	3	85	135 7	156	14 65	117 1	151 8	149 4	15 24	−32 1	−16 1	+ 6 6	−0.59

* Alcohol was given at the beginning of the second day in each experiment   The output is the total amount of substances in the urine, stools, and withdrawn blood

to offset the retention that occurred when the subject consumed the alcohol   One experiment (Table II) was prolonged for an additional day, and it was found that the sodium, potassium, and chloride loss approximately equaled the amount of these ions which had been retained during the period of alcohol consumption

The retention of potassium was reflected in the concentration of this ion in the plasma (Table V) While there was little or no change immediately following alcohol ingestion, there was a marked increase 8 hours later   The sodium and the chloride concentrations in the plasma were not elevated to a great extent, the largest increase noted was in Experiment 4 (Table II), where the sodium increase was 2 9 m eq and the chloride increase was 2 m eq   In the other experiments this change was not as much, but in each case there was at least an increase of 1 2 m eq

We were able to confirm the studies made by Himwich and coworkers (3) who found that after the ingestion of alcohol there was an increase in the blood sugar and lactic acid, and a decrease in the carbon dioxide content and the pH   In none of our experiments were the alterations as much as these workers observed

Nitrogen was retained in every case, but the positive balance is much more apparent in those experiments in which the sodium chloride intake was adequate, in each of these the amount retained was 2 grams or over, whereas it was less

than one gram when the sodium chloride intake was low   There was a reduction in the plasma proteins, which we believe was owing to an increase in blood volume (16)   An attempt was made to correlate the blood alcohol concentration with the changes in the water balance, but apparently no comparison could be made

DISCUSSION

All variables were controlled as far as possible in these experiments, but the activity of the subjects varied from day to day, and there was an increase in the activity during the period in which alcohol was given   It was observed that the respirations were increased in rate and depth after the alcohol was given, so that more water must have been lost through the expired air and by the increased amount of sweating during this period, than during any other period of the experiment No measurements were made of the water lost in this manner except that it was observed that more weight was lost during the period of drinking than could be accounted for by the water balance observed   Realizing these facts, we do not believe that a quantitative calculation can be made of the water exchange between the intracellular and extracellular phase

Darrow and Yannet (17) have estimated that the volume of extracellular fluids is approximately 27 per cent of the body weight   The extracellular fluid volume, however, may not remain

stationary when conditions of the experiment are altered In fact, it would appear that in the experiments reported here, there was an increase in the extracellular fluid volume. The retention of sodium and chloride without an increase in the plasma concentration and the increase in the blood volume (16) would indicate this increase. If, however, the assumption is made that the volume of extracellular fluid is approximately equivalent to 27 per cent of the body weight, then only approximately one-half of the retained potassium can be accounted for in this compartment (Table V) As pointed out above, this assumption is

TABLE V

A comparison of the potassium gain in the extracellular fluids with the gain in intracellular potassium

Experiment	Retained potassium during 8 hours after alcohol	Increase in plasma potassium concentration for same period	Total increase of potassium in extracellular fluid, assuming them to be 27 per cent of body weight	Gain of intracellular potassium	Intracellular fluid $\frac{K-0.017\,Na}{0.112}$
	m.eq	m.eq. per liter	m.eq	m.eq	cc.
1	50.3	1.1	19.1	31.2	275
2	56.8	1.9	37.4	19.4	171
3	40.0	4.5	112.5	72.5	−660
4	65.7	2.2	39.3	26.4	220
5	68.0	1.7	31.6	36.4	226

probably not correct in these experiments, however, we do not believe that the increase in the extracellular fluid volume was sufficient to account for all of the retained potassium This excess potassium must be retained in the cells As pointed out above there was a retention of nitrogen in all of the experiments with the exception of Experiment 3 Experiment 3 was of considerable interest in that the amount of retained potassium was not sufficient to account for the total increase of this ion in the extracellular fluids (Table V) However, this subject was somewhat overweight and his weight was not constant at the beginning of the experiment He was also in negative sodium and chloride balance Peters and Lavietes (18) have pointed out that the relation of body water to tissue solids does not remain constant during periods of changing nutrition It would appear then that in this subject there was a loss of potassium from the cells by actual destruction, since the gain in extracellular potassium was greater than was expected

from the amount retained by the body It is to be assumed, then, that since both potassium and nitrogen were retained in excess of the amount that was accounted for by the extracellular fluids, there was a gain in the intracellular fluids If the amount of retained potassium over that accounted for by the increase in the interstitial fluids is used as a basis for calculating the amount of retained intracellular water (19) then it is found that a slight increase in this compartment occurred in all experiments except Experiment 3 (Table V) Again, if the balance of intracellular water is calculated from the amount of retained nitrogen, as suggested by Darrow and Yannet (17), it is found that there is a discrepancy of approximately 100 cc. of fluid

If the retained sodium and chloride be calculated on the same basis, namely, assuming the extracellular fluids to be 27 per cent, it is found that only a very small amount cannot be accounted for in this partition It should be pointed out that potassium was retained in greater quantity than either the sodium or the chloride ions

It has been shown that when alcohol is given to dogs a diuresis occurs (1), and we have shown that in man a diuresis occurs in the first part of the drinking period, but that thereafter there is a retention of fluids It is to be noted that the concentration of sodium, chloride, potassium, and nitrogen in the urine was very much lowered so that the excess urine represents a water diuresis It would appear therefore that alcohol has an effect on the renal epithelium allowing the water to pass but holding back potassium in large amounts and sodium, chloride, and nitrogen in smaller amounts When the potassium and nitrogen are considered it appears that the effect of alcohol is not unlike that noted for the absence of the adrenal cortical hormone (20)

During the recovery phase, potassium, sodium, chloride, and nitrogen were lost in greater amounts than they were taken in Since the loss occurred when the concentration of alcohol in the blood had fallen to lower levels it would appear that the barrier to the excretion of these substances had been removed The excess of these ions was lost from the extracellular fluid and if it is assumed that a portion of the intracellular potassium exists in a diffusible form *'* would be

a loss of potassium from the cells   At the same time water was also lost

It is most unlikely that the shift of water to the intracellular phase is responsible for any of the symptoms resulting from acute alcoholism in man, since the amount of water gained by the cells was too small to bring about edema of any organ   In fact, the most severe symptoms that were noted occurred in Experiment 3, in which the calculated intracellular water was less during the recovery phase than in the beginning of the experiment, however, the plasma potassium concentration had increased to 8 3 m eq per liter

Potassium in excessive amounts is a depressant (21) and produces malaise, nausea, vomiting, and headache, all of which symptoms are observed after excessive alcohol ingestion   It is suggested that the increase in the concentration of plasma potassium after the ingestion of alcohol may be responsible for some of the after effects of over-indulgence in alcohol   In our experiments, the unpleasant symptoms of postalcoholic excess appeared at a time when the concentration of alcohol in the blood had fallen and when the potassium concentration was at its maximum

In all of the experiments reported here there was a slight increase in the level of the blood lactic acid, however, it seems unlikely that the slight increase noted would in any way be responsible for the acidosis produced or for any of the symptoms observed after overindulgence in alcohol

SUMMARY

1 Some of the effects of alcohol have been studied by means of balance experiments on healthy adult volunteers in which a constant food, fluid, and salt intake were given   Data from such studies reveal a marked retention of potassium, sodium, chloride, nitrogen, and water

2 It is suggested that the retention was produced by the direct action of alcohol on the excretory powers of the kidneys

3 An increase in the plasma potassium concentration occurred   Reasons are advanced for the belief that some of the symptoms observed following acute alcoholism in man are owing to an increase in the plasma potassium concentration

4 Previous work on the acid-base balance has been confirmed

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ESTIMATIONS OF THE WORK OF THE HEART DURING AND BETWEEN ATTACKS OF ANGINA PECTORIS

By ISAAC STARR, C. J. GAMBLE, J S DONAL, AND L. H COLLINS

(From the Departments of Research Therapeutics and of Pharmacology and the Medical Division of the Hospital of the University of Pennsylvania Philadelphia)

Angina pectoris is commonly attributed to a disproportion between the work demanded of the heart and the blood or oxygen supply available to it (1, 2, 3, 4, 5) That the heart's work increases during the attack of pain has been inferred from several well known facts First, increased blood pressure is common during the period of pain, although there is no complete correlation between the degree and duration of pain and the change in blood pressure (6, 7) Second, situations which commonly induce anginal attacks, such as exercise, excitement, food, and cold increase the cardiac output of normal subjects (8, 9)

The duration of most spontaneous attacks of angina following effort is too brief to permit satisfactory estimations of cardiac output during the pain Physicians have hesitated to induce attacks of cardiac pain of a duration sufficient to permit such estimations Probably these reasons account for the lack of estimations of cardiac output in the huge literature on angina pectoris Therefore the widely accepted view that angina is associated with increased heart work has never been verified by measurement

During a long series of estimations of cardiac output, carried out on patients during the last eight years, we had several unexpected opportunities to study angina pectoris These opportunities were seized Therefore we have secured satisfactory estimations of cardiac output, metabolic rate, blood pressure, pulse rate, and respiration, during cardiac pain in four patients Comparable estimations, when the patients were free of pain, were secured also

Three of our patients had typical angina pectoris Two have since died of their disease and in one a necropsy was secured The fourth individual suffered from atypical cardiac pain apparently induced by digitalis

In two instances we secured data on the physiological changes which accompanied the relief of pain after the administration of nitroglycerine.

In all of these patients the estimated work of the left ventricle was much larger during the pain than in its absence Our one necropsy proved the coronary circulation to have been impeded Therefore, our results support the generally accepted view of the causation of angina pectoris

METHODS

Estimations of cardiac output and metabolic rate were performed in the manner described (10 11) Left ventricular work has been calculated by the formula —
Work = cardiac output × mean blood pressure × 13.6 (12) Patient W B had slight aortic regurgitation and in this case the estimation of work is too small as the amount of blood leaking through the aortic valve is not included in the estimation of cardiac output The increased blood pressure during pain would tend to increase the error and although this would be somewhat offset by the decreased duration of diastole with the faster pulse rate, we believe that the actual increase of heart work during the pain must have been larger than our estimate of it.

Criteria discussed before (13) have permitted us to evaluate the significance of differences in cardiac output estimations in statistical terms.

The subjects were lying at rest 15 or more hours after their last meal Ward patients were brought to the laboratory in a wheel chair and lay on the bed over ½ hour before the first estimation The one outpatient (W B) lay down for 1 hour before the first estimation.

RESULTS AND DISCUSSION

The results in Case L. S, in which we have observations of blood pressure and pulse rate just before pain began, have been set forth in detail in Figure 1 This was our most intelligent subject, and he was able to give a clear account of the variations in intensity of his pain A very nervous individual the attack was probably induced by excitement

Table I gives the results secured in the other three patients The action of digitalis was perhaps a factor in the induction of attacks in Cases W B and A K. Estimations of cardiac output had been made previously on both these patients
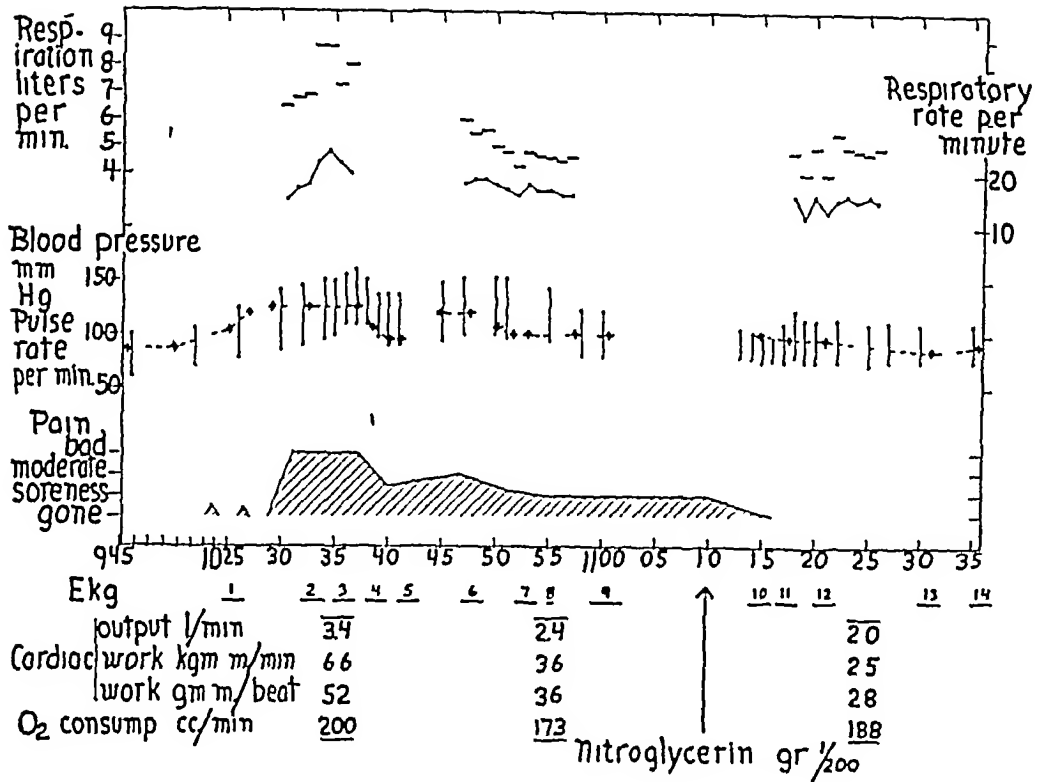
FIG 1 OBSERVATIONS MADE BEFORE, DURING, AND AFTER AN ATTACK OF ANGINA
PECTORIS IN CASE L S

The figures given for cardiac work include only that part contributed by the left ventricle

without causing them discomfort   In Case K P the attack was induced by adrenalin, given without knowledge that the patient had had angina previously

In every case the work of the left ventricle was greater during the pain than when the patient was comfortable   When these results are averaged, the increase of work during the pain is statistically significant   Increased blood pressure was a factor in the elevation of the heart's work in every instance   Cardiac output increased in three of the four cases   This increase is significant in each of these cases, when the difference is judged by the standards which we have set for ourselves (13)   In Case W B no change of cardiac output during pain was demonstrated, but the blood pressure rose to a level higher than that found in any other case

In Patients L S and K P estimations were made during both mild and more severe pain The results indicate that the greater the heart's work the more severe the attack, but we do not

have sufficient data to make the differences significant

Changes in oxygen consumption during the pain were usually small except after adrenalin, and when these results are omitted the average change is not significant

Respiratory volume was usually larger during pain, Case W B , after nitrites, being the only exception

The pulse rate was faster during pain in three of the four cases, the slowing in the remaining case was due to digitalis

After the pain had been relieved by nitroglycerine, the blood pressure and the heart's work were lower in both the patients to whom this drug was administered   After this drug the cardiac output was significantly lower in Case L S   It was elevated, but not significantly, in Case W B The pulse rate fell markedly in both instances, a result the opposite of that which usually follows the administration of nitroglycerine to normal subjects

TABLE I

Data obtained during and between attacks of angina pectoris

Case	Date and time	Cardiac output	Left ventricular work	Pulse	Blood pressure	Metabolic rate		Respiration		Remarks
								Volume	Rate	
		liters per minute	kgm. m. per minute	per minute	mm. Hg	cc. per minute	per cent	liters per minute	per minute	
W B.	May 6, 1933									
	9.45	3.7	4.6	76	140/45	229	+1.3	6.7	15	Luetic heart disease with aortic regurgitation. Not on digitalis. Comfortable
	10:00	4.3	5.4	72	140/45	229	+1.3	6.9	17	
	May 20 1933									
	9:54	4.2	7.0	92	190/35	204	−9.6	7.9	18	Anginal pain during this run. On digitalis since May 6, 1931
	10:34	4.8	6.2	73	150/40	260	+18.9	8.3	18	After relief by nitroglycerine grains 1/100 under the tongue
	May 29, 1933									
	10:00	2.9	3.5	72	140/40	209	−7.7	6.9	20	Comfortable—Off digitalis since last run
	10:15	3.0	3.5	72	140/40	209	−7.7	6.5	19	
K. P	Jan. 28, 1936									
	10:55	3.4	5.2	64	135/85	248	+11	5.3	9	Case of myxedema taking thyroid. Comfortable
	11:05	3.6	5.4	65	135/83	248	+11	5.4	13	
	11:25									Given 1 cc. epinephrine subcutaneously
	11.50	4.5	7.0	70	135/94	289	+30	6.7	12	Substernal pain slight
	12:09	5.4	9.0	95	175/72	319	+43	9.3	18	Substernal pain severe
	April 17, 1936									
	11:01	3.5	4.3	64	115/82	170	−23	4.5	8	Has had less thyroid since last run—No pain now
	11.10	2.8	2.9	66	119/83	167	−24	4.7	7	
A. K.	April 26, 1935									
	10:20	2.3	4.0	72	155/102	207	−20	6.4	12	In flutter since 1911 Not on digitalis. Comfortable
	10:30	1.9	3.3	72	155/100	215	−13	6.5	11	
	May 1, 1935									
	10:25	2.7	5.1	60	180/100	231	−11	7.1	18	Fully digitalized. Still in flutter Dull constant precordial pain throughout both runs
	10:35	2.7	5.1	60	170/106	239	−8	6.8	18	

SUMMARY AND CONCLUSIONS

In four cases of cardiac pain, three of them suffering from typical angina pectoris estimations of cardiac output, basal metabolic rate blood pressure, pulse rate, and respiration were made during the pain and, under comparable conditions, when the patients were free from it. In one case a necropsy was secured The changes following relief by nitroglycerine were studied in two cases

The results indicate that the work of the heart was significantly greater during the pain than when the patients were free from it

The results are consistent with the widely accepted view that cardiac pain is caused by situations demanding increased cardiac work when the heart's blood supply cannot be increased correspondingly

CASE REPORTS

Case L S A white man height 5 ft. 2 in. weight 107 pounds, age 50 in 1931 He had been well till 2 years before admission Then, while doing hard physical work he was seized by a severe attack of substernal pain lasting ½ hour Residual soreness for 3 days followed. After this attack he had frequent attacks of substernal "pain and pressure' on undue exertion, in ways less severe than the initial attack and lasting 5 to 10 minutes after cessation of activity Nitrites afforded prompt relief nitroglycerine was taken almost daily during the week prior to admission. Finally on October 3 1931, after a struggle with a refractory door in the outpatient department he had such a severe pain that he was sent to the ward

Physical examination revealed the following A nervous individual, substernal pain passing off hyperesthesia of upper portion of chest Pulse 104 blood pressure 132/90 Otherwise, examination was essentially negative. Orthodiagram—cardiac area 81 cm sq (normal) Electrocardiogram—QRS split in Lead II slurred in Lead III deep Q III T inverted in all leads

On October 9 1931 an estimation of cardiac output was planned Shortly after beginning to breathe through the mouthpiece he complained of substernal pain and coincidently blood pressure rose from 140/90 to 170/110 The estimation was abandoned and 0.3 mgm nitroglycerine administered with prompt relief

On October 12, 1931 a second attempt was made and Lead II of the electrocardiogram was connected also He had no pain on arrival at the laboratory but an attack developed during the inhalation of ethyl iodide, and the first estimation of cardiac output was made at its height. After this was over pain subsided but persisted during the second estimation. A third

tion was made after nitroglycerine had completely relieved him Figure 1 shows the results The electrocardiogram showed nothing of interest

He continued to have similar attacks in the Hospital On October 31 he had a severe one during a gastro-intestinal x-ray examination

On November 6, 1931, he was seized with a severe pain about midnight, nitrites failed to relieve it and morphine was required Electrocardiogram showed evidence of acute coronary thrombosis He died 12 hours later Necropsy was not permitted Diagnosis angina pectoris, coronary thrombosis

Case IV B A white man, 5 ft 6 in tall, weight 157 pounds, age 56 in 1933

In 1931, the Wassermann was found to be strongly positive, the heart was enlarged (orthodiagram plus 27 per cent) with signs of aortic regurgitation of moderate degree Blood pressure was 150/50 Antiluetic treatment was commenced and was continued, with intermissions, until his death

In 1932, he began to have attacks of severe substernal pain, radiating down both arms, and induced by exertion These attacks were relieved by nitroglycerine He soon became unable to work, having anginal attacks on slight exertion several times a week

On May 6, 1933, basal cardiac output was estimated uneventfully After this, he was put on 0 15 gram of digitalis daily

On May 20, 1933, a second estimation of basal cardiac output was performed after an hour's rest on the bed He complained of substernal pain soon after inhalation had begun, and it persisted without great severity till the first estimation was completed He was then given 0 6 mgm nitroglycerine under his tongue which relieved the pain and restored the blood pressure to its previous level in 5 minutes A second group of estimations was then performed Table I gives the results On conclusion he was told to discontinue digitalis

A month later another duplicate estimation of basal cardiac output was made without discomfort

During 1934, the anginal attacks grew steadily more frequent and severe One day he needed 27 tablets of nitroglycerine.

In January 1935, he was admitted to the ward in congestive failure He died a week later

Necropsy disclosed syphilitic aortitis and aortic valvulitis, hypertrophy and dilatation of the heart, an infarct of the lung, bilateral pleural effusions, and passive congestion of the abdominal viscera The aperture of the left coronary artery was narrowed so that a small probe was admitted with difficulty, the right aperture was normal The coronary vessels showed moderate yellow intimal thickening and were unobstructed

Clinical diagnosis Syphilitic heart disease with aortic valvulitis and regurgitation, angina pectoris, left coronary obstruction

Case K P A white man, born in Poland, was 5 ft 2 in tall, 156 pounds in weight and 50 years old in 1936 He was well till 1928 when he developed vague pains in his limbs diagnosed as arthritis, which persisted, with

numerous remissions, until admission in 1933 Deafness and failing vision were additional complaints Physical examination revealed a man of very low intelligence, an appearance suggestive of myxedema, infected tonsils, a boggy prostate, and signs of mild arthritis of right knee and of interphalangeal joint of right thumb The routine laboratory tests were negative. Basal metabolic rate was —5 per cent Thyroid was not given Tonsillectomy was performed, and the prostate massaged twice weekly He was discharged improved

In the summer of 1935 he began to complain of dyspnea on exertion, also of substernal pain on climbing stairs He returned to the Outpatient Department in December where he was found to be much confused mentally with a basal metabolic rate of —33 per cent. He was given thyroid and, not doing well, was sent to the ward

The patient was so dull that no history of angina was obtained on admission Physical examination was essentially as before Orthodiagram showed the heart to be 30 per cent larger than predicted. Electrocardiogram showed a PR interval of 0 16 second and small T waves in all limb leads

In ignorance of the presence of angina pectoris, the patient s cardiac output was estimated on January 28, 1936 After control estimations he was given 1 cc. of epinephrine subcutaneously and a second set of observations begun Substernal pain began before any samples had been taken, and the first estimation was made while it was mild but increasing The second estimation was made while pain was severe, and the blood pressure at its height The blood pressure and pain diminished after the estimation had been completed, but slight distress and some elevation of blood pressure were still present 45 minutes later He had no other attacks while in the Hospital

He was discharged on a larger dose of thyroid and soon felt much better, but late in March 1936, having a number of indefinite complaints, he was readmitted Basal metabolic rate was now —27 per cent On April 17th basal cardiac output was estimated again, uneventfully The results are given in Table I

Since discharge, he has continued under supervision of the Outpatient Department Therapy has been aimed both to keep him comfortable and to keep his metabolic rate low enough to prevent undue angina. This has been successfully accomplished

At date of writing (1938), he is doing light work and reports that he has only infrequent attacks of substernal pain, never severe enough to require nitrites Clinical diagnosis Myxedema, angina pectoris

Case A K A white man, 5 ft 11 in tall, 190 pounds in weight, age 59 in 1935

Hyperthyroidism and subtotal thyroidectomy in 1912 Auricular flutter was diagnosed by electrocardiogram in 1914 and has persisted to date During this period he was admitted to the ward repeatedly for headache, nervousness, palpitation, belching, constipation, and atypical precordial pain This pain was often present for several days at a time. A dull ache, it was never

severe, and did not radiate. No sense of constriction accompanied it. When present during rest, it was made much worse by exertion.

In 1935, increased shortness of breath led to his admission Physical examination showed slight peripheral arteriosclerosis, rales at lung bases, enlarged heart (orthodiagram plus 42 per cent), electrocardiogram showed flutter with block varying from 2 to 1 to 4 to 1 Blood pressure averaged 190/115 The ventricular rate varied from 130 to 60 X ray suggested luetic osteoperiostitis of skull left tibia, right fibula. The rales disappeared after a few days. The Wassermann was negative.

On April 26, 1935 the patient had received no digitalis for over a month. Duplicate estimations of basal cardiac output were performed uneventfully After this he was placed on digitalis.

By May 1, 1935 he was fully digitalized but was suffering from precordial pain as described above. This persisted throughout both duplicate estimations of cardiac output. Table I gives the results

After this date digitalis was discontinued. The pain soon disappeared, but the pulse became more rapid and irregular Digitalis was begun again but precordial pain returned almost immediately It was discontinued and relief followed again

He left the hospital soon after and has not been seen since. Clinical diagnosis Hypertensive cardiovascular disease auricular flutter atypical precordial pain

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CRITICAL REMARKS ON THE DETERMINATION OF URINARY EXCRETION OF ASCORBIC ACID

By M van EEKELEN and M HEINEMANN [1]

(From the Laboratory of Hygiene University of Utrecht Netherlands)

Szent-Györgyi (1) Svirbely and Szent Györgyi (2) and Tillmans (3) have demonstrated that ascorbic acid possesses reducing properties, and that it can be determined by titration with the redox-dye 2,6 dichlorophenol indophenol It therefore became possible to measure this vitamin in tissues and body fluids In urine, an indophenol reducing substance was demonstrated by

van Euler and Klussman (4), van Eekelen *et al* (5), and Harris *et al* (6) Harris *et al* (6), van Eekelen *et al* (7), and Johnson and Zilva (8) have shown that following the intake of large amounts of ascorbic acid the urinary excretion of reducing substances increases Application of the reduction reaction to the quantitative determination of ascorbic acid requires that other reducing substances be removed or prevented from reacting by controlling the conditions Titration in an acid medium excludes ferrosalts and gluta

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[1] At present Research Fellow in the Department of Internal Medicine, Yale University School of Medicine.
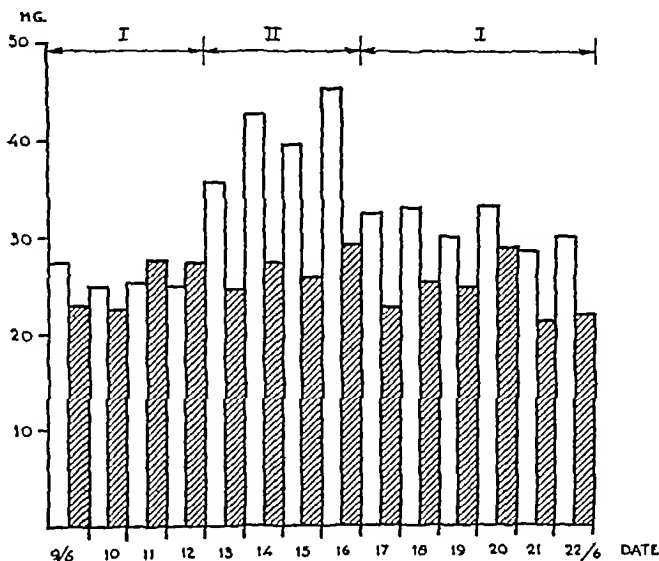
FIG 1 THE INFLUENCE OF DIETARY PROTEIN ON THE EXCRETION OF NONSPECIFIC REDUCING SUBSTANCES

mgm. cevitamic acid in urine after precipitation with mercuric acetate, in 24 hours.

total reducing substances in 24 hours expressed as mgm. cevitamic acid.

I   low protein diet.
II high protein diet.

293
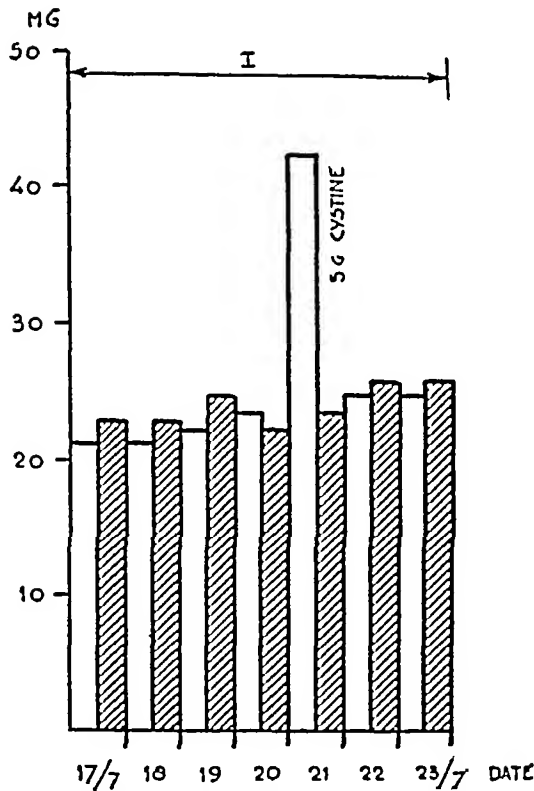
FIG 2   THE INFLUENCE OF CYSTINE ON THE EXCRETION
         OF NONSPECIFIC REDUCING SUBSTANCES

|///////| mgm cevitamic acid in urine after precipita-
         tion with mercuric acetate, in 24 hours

| | total reducing substances in urine expressed
         as mgm cevitamic acid, in 24 hours
    I   low protein diet

thione (10, 11, 12)   Other substances, e g , cys-
teine, ergothionine, thiosulphate, interfere even in
an acid medium   These substances can be re-
moved by precipitation with mercuric acetate (13,
14, 15) or with barium acetate (16)   Some ex-
periments will be presented demonstrating the
magnitude and the variability of the errors which
may be incurred if these interfering substances
are not removed   Furthermore, the technique of
the saturation test will be discussed

Since our method has not been described in
the American literature, it will be given in some
detail

METHOD

Reagents   One hundred mgm of 2,6 dichlor-
ophenol indophenol (Hoffman-LaRoche) are dis-
solved in 500 cc of distilled water at 85° C,
filtered, and approximately 0 2 gram of $NaHCO_3$

added   This solution is standardized against
crystalline ascorbic acid (levo-ascorbic acid
Roche) and is fairly stable for a period of a few
weeks if kept in a cool dark place   It must, how-
ever, be standardized once a week   In the course
of a month we have noted a decrease in concen-
tration of not more than 5 per cent

Mercuric acetate, 20 per cent solution, filtered,
after standing 2 days, to remove Hg (OH)$_2$
formed by hydrolysis

Trichloroacetic acid 3 per cent and 10 per cent
    Procedure   Freshly voided urine[2] has been
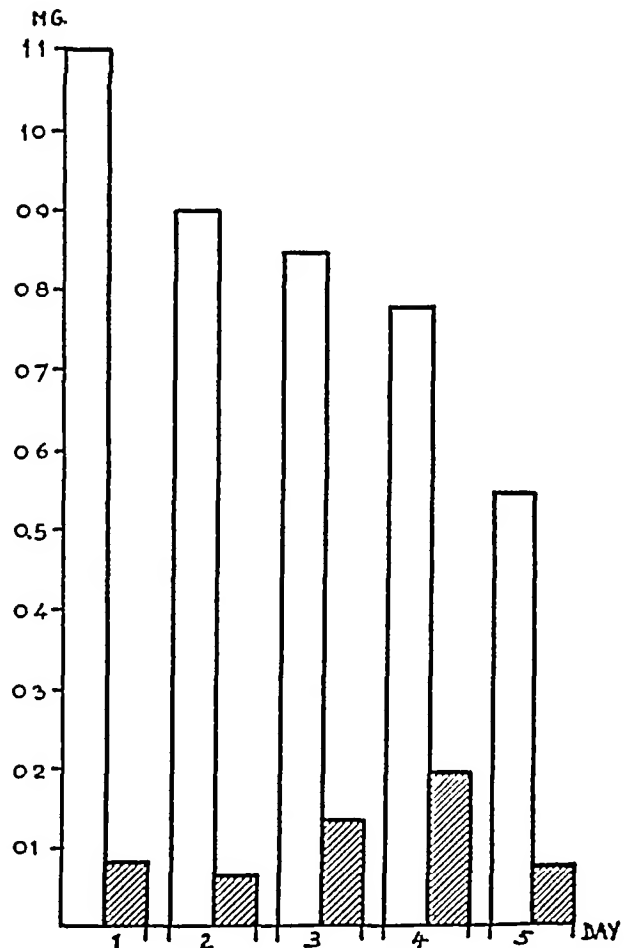
FIG 3   THE EXCRETION OF NONSPECIFIC REDUCING
         SUBSTANCES BY A DIABETIC PATIENT

|///////| mgm. cevitamic acid per ml of urine after pre-
         cipitation with mercuric acetate.

| | total reducing substances per ml urine ex-
         pressed as mgm cevitamic acid

[2] No satisfactory method is available to prevent loss of
ascorbic acid from urine on standing (8, 21, 22)

titrated with and without the removal of interfering substances in human subjects under various conditions   In the direct titration of urine without preliminary removal of nonspecific reducing substances, 1 or 2 cc. of urine are acidified with 5 cc. of 3 per cent trichloroacetic acid and titrated at once   Titration is carried out from a microburette containing the dye into a white porcelain evaporating dish, which contains the aliquot to be assayed

To eliminate interfering substances, 20 cc. of the mercuric acetate solution are mixed with 10 cc. of freshly voided urine in a 40 cc. centrifuge tube and centrifuged for 2 minutes   H S is immediately bubbled through the decanted supernatant fluid   The time elapsing between addition of mercuric acetate and treatment with H_2S should not exceed 10 minutes in order to avoid irreversible oxidation of the vitamin   Treatment with H_2S is continued until no more mercuric

sulphide is formed from the surplus mercuric acetate in the solution   The solution is then filtered, resaturated with H_2S, stoppered with a cork and kept in a dark place for at least 6 hours Thereafter, the H_2S is removed with a continuous stream of O_2 free nitrogen or carbon dioxide, until lead acetate paper does not darken   Five cc. of this filtrate is titrated after adding 1 cc of 10 per cent trichloroacetic acid   With urines containing large amounts of ascorbic acid, a smaller aliquot is taken, *e g*, 1 cc, in which case 5 cc of 3 per cent trichloroacetic acid are added

Solid barium acetate has been used to remove interfering reducing substances in place of mercuric acetate solution   thus eliminating the need for H_2S In general, the values obtained by this method, if it is carried out immediately after the urine has been voided, agree closely with those found after mercuric acetate precipitation.

Saturation test   After depletion of ascorbic

Fig 4   The Excretion of Nonspecific Reducing Substances by a Tuberculous Patient

A and *B* represent different saturation tests

6-hour urinary excretion of mgm. cevitamic acid after precipitation with mercuric acetate.

6 hour urinary excretion of total reducing substances expressed as mgm. cevitamic acid.

FIG 5 THE EXCRETION OF NONSPECIFIC REDUCING SUB-
STANCES BY A PATIENT WITH PEPTIC ULCER DURING
A SATURATION TEST

[////] 6-hour urinary excretion of mgm cevitamic
acid, after precipitation with mercuric acetate

[   ] 6-hour urinary excretion of total reducing sub-
stances expressed as mgm cevitamic acid

given by parenteral route might cause a transitory
increase in urinary excretion, not because the
tissues are truly saturated, but because even un-
saturated tissues require time to take up the
vitamin   This is especially true if the vitamin is
given intravenously, since in this case the con-
centration in the blood is elevated so suddenly that
a transitory overflow into the urine simulates a
peak of saturation   That this is true is corro-
borated by the observation that the same dose
given per os the next day causes no surplus ex-
cretion (17)   Contrasting the various methods
of administration, Hawley and Stephens (18)
and Heinemann (19) have shown the surplus
output (in a saturated subject) during the first
6 hours following intake per os to be about 50
per cent of the 24 hour excretion   After sub-
cutaneous injection, approximately all of the sur-
plus excretion occurs in 6 hours   Whereas, fol-
lowing intake per os, the rise in urinary excretion
takes place in the second 3 hours of the 6-hour
period, it is observed in the first 3 hours following
subcutaneous injection (20)   The quantity of
ascorbic acid given also influences the rise of con-
centration in the blood   A very large dose, e g ,
1000 mgm as a single dose per os, may induce
phenomena similar to those observed after intra-
venous administration   For this reason most in-
vestigators never use a dose greater than 300
mgm at one time

Saturation tests, therefore, should be carried
out by giving ascorbic acid per os or, for special
purposes, subcutaneously, and in modest daily
doses   Intravenous   administration   of   even
modest doses or intake by mouth of an excessively
large dose at one time can simulate saturation by
causing a urinary surplus excretion before the
depletion of the body has been overcome

Examples demonstrating the independence be-
tween total reducing capacity of the urine and
that resulting from ascorbic acid only are given
below   In order to simplify the technique of
saturation tests, the procedure previously fol-
lowed, observation of urinary excretion for a
period of 24 hours, has been altered in the follow-
ing way (19)   immediately after voiding at 9 a m,
ascorbic acid is given and its concentration in the
urine is determined in samples voided at 12 p m
and 3 p m respectively

acid repeated doses of this vitamin can be given
without any increase in urinary excretion until
the deficiency has been completely corrected
The point at which urinary excretion increases
under these circumstances is taken as the satura-
tion point   At this point the ascorbic acid con-
centration in whole blood is quite regularly 14
mgm per liter (17)   The dose necessary to pro-
duce this saturation and a consequent increase in
urinary output is inversely proportional to the
amount of the vitamin stored in the body
Therefore, the saturation test has the practical
importance of demonstrating various degrees of
hypovitaminosis, the effects of administered as-
corbic acid varying with the degree of saturation
or depletion of the organism concerned

These effects depend, too, on the rapidity of
absorption   In an organism depleted of ascorbic
acid, the capacity of the tissues to take up ascorbic
acid from the blood stream may, following intake
per os, be so great that moderate doses are ab-
sorbed completely without increase of urinary
excretion   In the same subject, a similar dose

Subjects on a high protein diet or after intake
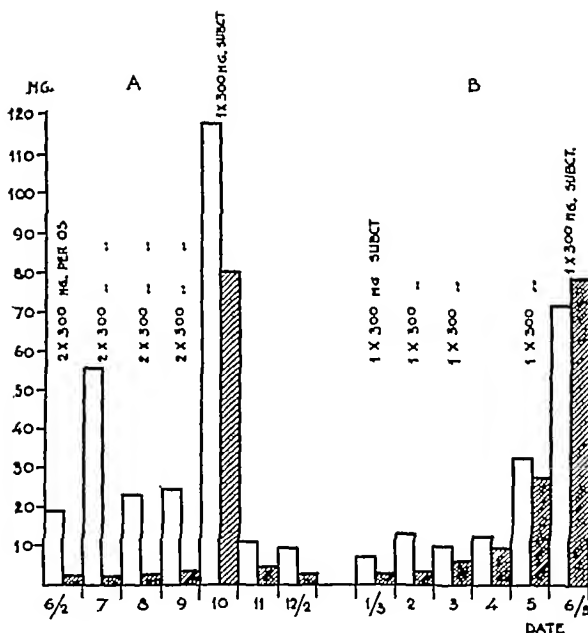
Fig. 6   The Excretion of Nonspecific Reducing Substances by a Patient with Peptic Ulcer during Two Different Saturation Tests A and B

6-hour urinary excretion of mgm. cevitamic acid after precipitation with mercuric acetate.

6-hour urinary excretion of total reducing substances expressed as mgm cevitamic acid.

of cystine excrete a large quantity of reducing substances even though the ascorbic acid intake is maintained at a constant level (21)   In Figures 1 and 2 the error of direct titration of urine is demonstrated, the tall blank columns indicating total reducing substances and the dark columns the total amount of ascorbic acid after mercuric acetate precipitation   In certain pathological conditions (diabetes (Figure 3), tuberculosis (Figure 4), peptic ulcers (Figures 5 and 6)) the excretion of large amounts of unspecific reducing substances other than ascorbic acid has been observed   Even in these diseases the excretion of reducing substances other than ascorbic acid has been observed to vary largely from patient to patient and in the same individual from day to day

It so happens, that in Figure 4A the total reducing capacity parallels the amount of ascorbic acid excreted, in Figure 4B a second saturation of the same patient has been carried out and an increase in the output of total reducing substances is observed after a dose of 1500 mgm of ascorbic acid while surplus output of ascorbic acid occurs only after an intake of 2100 mgm   A similar observation is given in Figure 6A (increased total reducing capacity after 900 mgm) and Figure 6B (parallelism between results of direct titration and that after mercuric acetate precipitation)

From the foregoing examples it follows that the total reducing capacity of urine, estimated by direct titration can be very high while ascorbic

acid is found in normal amounts   This high total reducing capacity is chiefly due to thiosulphate (16), a fact on which the method of its elimination with barium acetate is based

In connection with these observations there are certain other problems that must be discussed Harris *et al* (23), Abbasy *et al* (24), Youmans *et al* (25) claim that healthy people, under normal nutritional conditions (with a sufficient supply of vitamin C, but not saturated), excrete daily urinary amounts which are nearly constant Based on these observations, a normal level of urinary output is supposed, excretion below this level consequently is considered as demonstration of deficiency   These levels are based on direct titration, which, as has been shown above, cannot be regarded as reliable   In a number of observations we have noticed that the urinary output of ascorbic acid, estimated after precipitation by mercuric acetate, declined when no vitamin C was taken   On the other hand, when only 60 per cent of the daily dose required to maintain saturation was taken, no decrease in urinary output could be observed in spite of decreasing ascorbic acid content of the blood (22)   Furthermore, the daily urinary output can differ under normal dietetic conditions, one of us having an average excretion of 12 mgm, the other of 23 mgm daily (after precipitation with Hg—acetate or Ba—acetate) Cognizant of the fact that even in the same individual daily fluctuations can occur, it would be difficult, if not impossible, to distinguish a "normal level" of urinary excretion

Biological assay indicates that the substance causing increased reducing power of the urine after massive doses of ascorbic acid is indeed ascorbic acid (9)   Whether or not the small normal excretion actually represents ascorbic acid has not been established yet and seems of little importance for the purposes of our studies

SUMMARY

Ascorbic acid in urine cannot be determined reliably by direct titration with 2,6 dichlorophenol indophenol   Reducing substances other than ascorbic acid, present in urine, also decolorize this indicator   They can be removed by precipitation with mercuric acetate   The excretion of these interfering substances can increase considerably,

under various conditions, and independently of that of the vitamin

The technique and significance of saturation tests have been discussed

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THE GUANIDINE BASES IN THE BLOOD OF DOGS WITH EXPERIMENTAL HYPERTENSION PRODUCED BY CONSTRICTION OF THE RENAL ARTERIES

By CHARLES G CHILD

(From the Department of Experimental Surgery of the New York Hospital and Cornell Medical College New York City)

When Goldblatt and coworkers (1) in 1934 first announced that a significant and sustained elevation in arterial blood pressure could be produced in dogs by constriction of the renal arteries they also reported a few experiments in which the level of the guanidine bases in the blood stream of these animals had been followed Although their preliminary studies indicated that these substances were not involved in either the production or maintenance of this type of experimental hypertension, they suggested that the results were not conclusive and that the group as a whole deserved further study It is the purpose of this report to record the results of several experiments which have been undertaken in this laboratory in an effort to determine what relationship, if any, these bases bear to this type of hypertension.

That there might be some relationship between the guanidine bases and hyperpiesia is not a particularly new concept In 1924 Major and Stephenson (2) emphasized that many of these substances produced a marked and prolonged rise in blood pressure Following Major's initial studies several efforts have been made to demonstrate an increased amount of the guanidine bases in the blood of hypertensive patients (3) as well as in eclampsia (4) That these investigations have been inconclusive is evidenced by the contradictory nature of the reports that have appeared up to the present time. Of further interest are the experiments of Minot and Dodd (5) who report that an accumulation of guanidine bases is present in the blood stream of dogs in which a ricin necrosis has been produced Therefore not only because of the normal existence of these substances and their excretion by the kidney but also because of their relationship to degenerating tissue it has seemed significant to establish what relationship, if any, they might bear to that type of experimental hyper

which may be produced by constriction of the renal artery

METHODS

Male and female dogs weighing between 10 and 12 kgm. have been used throughout the experiments The daily determinations of blood pressure have been made either by the Van Leersum carotid loop method or in several of the shorter experiments by direct arterial puncture. Constriction of the renal artery has been obtained by either the Goldblatt clamp or small silver clips designed in this laboratory complete occlusion of the artery has been accomplished by ligature with heavy silk. The blood urea nitrogen and nonprotein nitrogen have been determined by the usual methods. The level of the guanidine bases has been determined by the colorometric method of Major as modified by Minot and Dodd (5) These chemical studies have been made in all cases on blood withdrawn from the jugular vein at least eighteen hours after the last intake of food. All animals have been fed a normal balanced diet.

The experiments which have been undertaken are given below

Experiment I Severe hypertension was produced in two dogs with but a single kidney and that transplanted to the femoral vessels This was accomplished by constricting the femoral artery with a small silver clip Both of these animals died and at autopsy diffuse necrosis of the kidney was found in each instance. The

TABLE I

Experiment I

Dog number	Date	Blood pressure	Blood urea nitrogen	Guanidine base
	1937	*mm Hg*	*mgm per 100 cc*	*mgm per 100 cc*
SA-37 2ad	April 29	150	18 0	0 6
	April 29	Constriction of the artery		
	May 10	180		
	May 13	230	70 0	4 3
	May 14	160		5 0
	May 14	Death of animal		
SA-37-3ad	May 5	140	20 4	0 6
	May 6	140	18 0	
	May 6	Constriction of the artery		
	May 7	150		? 4
	May 8	170		
	May 9	210	55 9	?
	May 9	Death		

blood pressure, blood urea nitrogen, and guanidine base values are given in Table I

Experiment II A marked rise in blood pressure was produced in three normal animals by constriction of both renal arteries, and in one dog with a single kidney which had been transplanted to the femoral vessels by constriction of the femoral artery In all three of these a persistent hypertension was produced The values are given in Table II

TABLE II

Experiment II

Dog number	Date	Blood pressure	Non protein nitrogen	Blood urea nitrogen	Guanidine base
	1937	*mm Hg*	*mgm per 100 cc*	*mgm per 100 cc*	*mgm per 100 cc*
SA-37-75	May 13	130			
	May 14	145		17 7	0 6
	May 14	Constriction of both renal arteries			
	May 16	160			
	May 17	130		18 6	0 6
	May 24	130			
	May 25	130		18 6	0 8
	May 25	Further constriction of both renal arteries			
	May 26	190			
	May 27	200			
	May 28	200	46 6	22 8	0 7
	June 4	210	44 1	22 3	0 8
	June 14	200		16 3	0 8
A-37-81	April 28	160		18 8	0 5
	April 28	Constriction of both renal arteries			
	May 12	240		34 0	2 1
	May 18	260		32 1	2 5
	May 21	240		30 2	1 6
	May 26	230	53 4	32 6	1 8
	June 2	210	39 9	22 3	0 8
	July 16	200			
SA-37-73	May 6	130		19 0	0 6
	May 6	Constriction of femoral artery			
	May 12	230		34 0	0 4
	May 17	160		25 1	0 4
	May 21	180		24 2	1 5
	May 25	170		20 0	0 8
	June 10	180		18 6	0 9
	June 16	170		20 9	0 9

Experiment III Two animals have been studied in which there had been an elevated blood pressure for over six months As the initiation of the hypertension in these animals antedated these experiments no normal values are available (Table III)

Experiment IV In an attempt to gain a nearer approach to the significance of the guanidine bases four animals were studied in which one renal artery was ligated In two of these the ligature was removed after a few days in an effort to promote absorption of necrotic renal tissue.

Experiment V As there seemed little doubt but that the values for the guanidine bases paralleled those obtained for the blood urea nitrogen and, when determined, the nonprotein nitrogen, it seemed of significance to con-

TABLE III

Experiment III

Dog number	Date	Blood pressure	Blood urea nitrogen	Guanidine base
SA-36-55	*1936*	*mm Hg*	*mgm per 100 cc*	*mgm per 100 cc*
	Oct 20	110		
	Oct 23	Constriction of both renal arteries		
	1937			
	June 9	220	16 7	0 6
	June 11	220	14 9	0 6
SA-36 34	*1936*			
	Sept 8	120		
	Sept 9	Constriction of both renal arteries		
	1937			
	June 11	190	13 0	0 6
	June 15	190	17 2	0'5

TABLE IV

Experiment IV

Dog number	Date	Blood pressure	Non protein nitrogen	Blood urea nitrogen	Guanidine base
	1937	*mm Hg*	*mgm per 100 cc*	*mgm per 100 cc*	*mgm per 100 cc*
SA-37-52	June 1	108	33 3	17 9	0 6
	June 1	Ligature of the left renal artery			
	June 3	124	34 2	17 9	0 7
	June 4	160	34 2	15 8	0 7
	June 5	178	38 0	13 0	0 6
	June 7	140	34 2	13 5	0 8
	June 7	Removal of ligature			
	June 8	122	27 2	12 5	0 7
	June 10	106		15 8	0 8
SA-37-70	May 24	160			
	May 25	160			
	May 26		36 0	17 7	0 7
	May 26	Ligature of the left renal artery			
	May 27	180	39 9	20 0	1 9
	May 28	180	57 0	30 7	2 1
	May 29	195	66 0	37 7	2 2
	May 29	Removal of ligature			
	May 30	160		16 3	2 2
	May 31	160		22 3	2 1
	June 1	150			
	June 2	160	65 1	33 0	2 7
	June 3	160	57 0	27 9	2 5
	June 4	160	60 0	29 8	2 3
	June 8	155	44 4	21 4	0 8
	June 14	155		22 3	0 8
SA-37-73	June 7	106	29 5	14 4	0 6
	June 7	Ligature of the left renal artery			
	June 8	102	32 1	13 0	0 6
	June 9	100	27 9	12 5	0 5
	June 10	100		25 6	0 5
	June 11	128		24 6	0 5
SA-37-74	June 7	98	36 3	17 7	0 7
	June 7	Ligature of the left renal artery			
	June 8	132	48 0	20 5	0 7
	June 9	120	37 5	15 3	0 8
	June 10	134		19 5	0 7
	June 11	146		17 7	0 7
	June 14	110		15 6	0 7
	June 16	116		16 3	0 7

trol these experiments by performing similar determinations on bilaterally nephrectomized dogs

DISCUSSION

In analyzing the above results as they appear under their various headings, one fact of particular significance is evident, that in general the rise in guanidine bases parallels the nitrogen retention in the blood stream irrespective of the blood pressure Dogs SA–37–61 and SA–37–77 in which bilateral nephrectomy was undertaken show a rise in guanidine bases comparable to Dogs SA–37–2ad and SA–37–3ad which died following restriction of the renal blood supply No

TABLE V

Experiment V

Dog number	Date	Blood pressure	Non protein nitrogen	Blood urea nitrogen	Guanidine base
	1937	*mm Hg*	*mgm per 100 cc.*	*mgm per 100 cc.*	*mgm per 100 cc.*
SA–37–61	May 24	130		17 7	0 6
	May 24	Bilateral nephrectomy			
	May 25			35 8	0 5
	May 26		150 0	83 8	3 0
	May 27	136	211 5	109 9	3 7
	May 28	126	264 0	138 8	4 1
	May 29		345 0	183 1	5 1
SA-37 77	June 2	116	32 4	17.2	0 6
	June 2	Bilateral nephrectomy			
	June 3		75 0	39 6	1 4
	June 4	104	155 7	75 9	3 1
	June 5	81	222 0	94 0	5 0

hypertension appeared in the nephrectomized animals, while the rise was marked in those in which the femoral arteries were constricted The question may be brought up whether or not these latter two animals merely died from renal insufficiency Undoubtedly this played a part, but their death was associated with a marked degree of hypertension, a phenomenon not shown by animals dying in total renal insufficiency such as is demonstrated by nephrectomized dogs

In the experiments in which an acute rise in blood pressure was produced by constriction of the renal artery and following which the animals survived, Dog SA–37–75 showed an insignificant transient rise in blood pressure following the first constriction without any change in the blood urea nitrogen, the nonprotein nitrogen, or the guanidine bases Following the further constriction of the arteries, there was a marked rise in blood

pressure accompanied by but slight rise in nonprotein nitrogen, blood urea nitrogen, and guanidine bases In Dog SA–37–81, however, there was a marked rise in blood pressure, moderate rise in nonprotein nitrogen, blood urea nitrogen, and guanidine bases These two experiments are compatible, within the limits of experimental error, with a conclusion that any rise in guanidine bases is related merely to the degree of nitrogen retention In Dog SA–37–73 a contradiction is found to this conception for here is an animal in which an elevated blood urea nitrogen is associated with an increased blood pressure while the guanidine bases are normal In fact, as the blood pressure and blood urea nitrogen fall, there is even a slight rise in guanidine bases This animal cannot be justly compared with the first two for the experimental situations differ somewhat this animal possessed but one kidney and that transplanted Although this theoretically should be of little significance, still it vitiates comparison

When the two animals with hypertension of long standing six and eight months respectively, are compared, the blood urea nitrogen and nonprotein nitrogen and guanidine bases are found to be normal, yet both of these animals show elevations in blood pressure far above their normal level

In the last group in which a correlation between the absorption of necrotic renal tissue, hypertension, and the guanidine bases was attempted, the results are contradictory In three, Dogs SA–37–52, SA–37–70, and SA–37–74, ligature of the renal artery was followed within twenty four hours by a rise in blood pressure In Dog SA–37–73 no rise appeared, at least not until late in the course of the experiment. In Dogs SA–37–52, SA–37–73 and SA–37–74 there were no significant changes in the blood urea nitrogen, nonprotein nitrogen, or guanidine bases But in Dog SA–37–70 all of these three factors showed a moderate transient rise. No explanation can be given for this other than to suggest that this animal experienced some difficulty in rapidly accommodating itself to the functional removal of one kidney It will be noticed that these values returned to normal after thirteen days

In Dogs SA–37–52 and SA–37–70, the ligature was removed from the artery in an attempt to promote a more rapid absorption of n

tissue No unusual results of this procedure were noted, as reflected in either the blood pressure, the blood urea nitrogen or the guanidine bases

CONCLUSIONS

1 The guanidine bases in the peripheral blood stream rise following bilateral nephrectomy

2 The guanidine bases rise in the peripheral blood stream in animals in which both arteries are partially constricted This rise is roughly proportional to the nitrogen retention, and apparently depends on the degree of renal damage occasioned by constriction of the artery

3 The guanidine bases do not rise in the blood stream following ligature of one renal artery, unless a nitrogen retention appears, which in turn is apparently dependent upon the occasional inability of the normal kidney to take over immediately the function of the opposite organ

4 No etiological relationship could be demonstrated between this type of experimental hypertension and the appearance of the guanidine bases in the blood stream occasioned either by partial constriction of one or both renal arteries, or by ligature of one renal artery

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THE EFFECT OF VITAMIN D ON CALCIUM AND PHOSPHORUS METABOLISM, STUDIES ON FOUR PATIENTS

By FULLER ALBRIGHT and HIRSH W SULKOWITCH

(From the Medical Service of the Massachusetts General Hospital and the Department of Medicine of Harvard University Medical School Boston)

There are many questions which are still obscure concerning the action of vitamin D One of the most important is whether the decrease of the calcium and phosphorus in the feces resulting from its administration is the result of increased absorption or of decreased reexcretion This is difficult to answer unless one administers calcium or phosphorus intravenously Whether the action is primarily on the calcium metabolism with secondary changes in the phosphorus metabolism or *vice versa* is also unsettled It is likewise uncertain whether the parathyroid glands play any part in the metabolic changes following vitamin D administration The present studies were undertaken to answer these and other questions

The data come from metabolic studies on four patients The first patient was a boy of fourteen years with a form of rickets resistant to vitamin D therapy His case history was reported by Albright, Butler, and Bloomberg (1) The essential features were that in spite of what would be usually considered as adequate vitamin D therapy he had had rickets all his life, that a bone biopsy showed that the condition was actually rickets with wide osteoid seams, that the abnormalities in his calcium and phosphorus metabolism were in the same direction as those in ordinary infantile rickets namely a normal serum calcium level a low serum inorganic phosphorus level, a high serum phosphatase level and an increased excretion of calcium and phosphorus in the feces, that the usual doses of vitamin D had no effect on these abnormalities, that, however, massive doses of vitamin D such as 45 cc. of viosterol (= 450,000 I U (International Units)) daily did correct the abnormalities With these large doses six changes resulted the serum calcium and phosphorus levels were elevated the fecal calcium and phosphorus excretions were decreased the urinary calcium excretion was increased, and the urinary phosphorus excretion was decreased One further point of interest to

the present discussion came out of the data of another article (1) The removal of one hyperplastic parathyroid gland resulted in a prompt elevation in the depressed serum inorganic phosphorus level and a fall in the serum calcium level This will be discussed below

The second, third, and fourth patients were all young individuals with idiopathic hypoparathyroidism Their case histories will appear elsewhere (2)

Metabolic data on Patient 1 (M G H Number 325 488) with rickets resistant to vitamin D

In Table I are shown some data obtained during this patient's fourth admission to the Massachusetts General Hospital The main purpose of the study was to determine whether vitamin D affects the phosphorus metabolism by increasing the absorption of phosphate from the gastro-intestinal tract or by decreasing its reexcretion The plan was to give a large part of the phosphate intravenously without vitamin D administration during the control periods, to repeat with large doses of vitamin D during the study periods, and then with a continuation of the vitamin D to give the phosphate by mouth

During the first two three-day periods the patient was placed on a low calcium moderately low phosphorus diet The results were quite surprising Not only were the fecal calcium excretions not increased at the expense of the urinary excretions (1), but the urinary calcium excretions were excessively high The explanation, in all probability, of this change is that it represents an after-effect of previous vitamin D therapy Until five days before the investigation started, he had been taking 2 cc. of 2500 D viosterol daily (200 000 I U) Another factor may have been that his bones were much less rachitic by that time owing to previous treatment His phosphatase level in the serum was still high however, 16 Bodansky units

TABLE I

Metabolic data on Patient 1 with rickets

Three day period	Calcium				Phosphorus				Serum			Therapy
	Urine	Feces	In-take	Bal-ance	Urine	Feces	In-take	Bal-ance	Calcium	Phos-phorus	Phos-phatase	
	grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc	mgm per 100 cc	Bodansky units	
1	0 61	0 08	0 30	—0 39	1 69	0 45	1 78	—0 36				Low calcium diet
2	0 64	0 08	0 30	—0 42	1 69	0 39	1 78	—0 30	10 9(III)*	3 0	16 2	Low calcium diet
3	0 77	0 18	2 40	+1 45	1 17	0 36	1 78	+0 25				Same plus calcium lactate p o
4	0 77	0 91	2 40	+0 72	1 11	0 58	1 78	+0 09				Same plus calcium lactate p o
5	0 73	0 95	2 40	+0 72	1 02	0 63	1 78	+0 13				Same plus calcium lactate p o
6	0 60	1 10	2 40	+0 70	2 47	0 50	3 51	+0 54	9 3(I) 10 7(II)	2 8 2 6	18 3 24 7	Same plus phosphate i v
7	0 48	1 16	2 40	+0 76	2 25	0 61	3 51	+0 65	10 1(I)	2 8	17 3	Same plus phosphate i v
8	0 43	0 86	2 40	+1 11	2 23	0 54	3 51	+0 74	(I)	2 6	20 0	Same plus vitamin D
9	0 87	0 35	2 40	+1 18	2 14	0 37	3 51	+1 00	9 6(III)	3 1	15 7	Same plus vitamin D
10	1 09	0 25	2 40	+1 06	2 35	0 39	3 51	+0 77				Same except phosphate p o
11	1 29	0 21	2 40	+0 90	2 36	0 36	3 54	+0 82	11 7(I)	3 6	14 8	Same except phosphate p o
12	1 35	0 33	2 40	+0 72	2 41	0 54	3 54	+0 59	11 6(I)	4 0		Same without vitamin D
13	1 30	0 25	2 40	+0 85	2 38	0 29	3 51	+0 84	12 1(I)	4 0	12 1	Same without vitamin D
14	1 15	0 40	2 36	+0 81	2 54	0 37	3 41	+0 50				Same without vitamin D
15	1 09	0 85	2 40	+0 46	2 45	0 75	3 51	+0 31	11 2(II)	3 8	14 9	Same without vitamin D

* Roman numeral indicates on which day of period blood determination was done

Periods 3, 4 and 5 differed from 1 and 2 in that 700 mgm of calcium in the form of calcium lactate were given daily by mouth As will be seen from Table I, most of this was absorbed and only a small part was excreted in the urine, the calcium balance becoming markedly positive The fecal phosphorus was increased and the urinary phosphate excretion was markedly lowered The blood values remained unaltered It should be noted in passing that up to three weeks after the last viosterol treatment there was evidence of excellent absorption of calcium This shows that the patient's resistance to vitamin D therapy was not caused by a rapid destruction of the vitamin

Periods 6 and 7 differed from the previous three in that he received 575 mgm of phosphate intravenously daily The fecal phosphorus excretion was not increased by this procedure, but the urinary phosphate excretion was markedly

elevated There was considerable increase in the retention of phosphorus, and the urinary calcium excretion was decreased These data suggest that excretion of phosphorus into the gastro-intestinal tract was not influenced by the pouring of a large amount of phosphate into the blood and was probably a very small factor, if existent at all, in this individual

Periods 8 and 9 differed from the previous two in that the patient received 20 cc of crystal-line vitamin D in propylene glycol three times daily (= 600,000 I U) There was a decrease in the already low fecal phosphorus excretion, without an increase in the urinary phosphate excretion, and, therefore, an increase in the phosphorus balance Inasmuch as reexcretion seems practically ruled out in this case (*v supra*) it seems certain that this change was a result of increased absorption The fecal calcium excre-

tion was markedly decreased and the urinary calcium excretion was considerably increased

Periods 10 and 11 differed from the previous two only in that the 575 mgm of phosphate which he was getting daily intravenously were given by mouth This caused no definite change in the phosphorus excretions, there being no rise in the fecal phosphorus values Thus the fecal phosphorus level under the conditions of this experiment was not affected when phosphate was given intravenously or when it was given by mouth, it was increased when calcium was given by mouth and it was decreased by the administration of vitamin D The fecal calcium values, furthermore, were not increased by the giving of phosphate by mouth but continued their downward trend started in Period 8 with the administration of vitamin D Thus the fecal calcium excretion was not affected by the phosphate in the diet, it was increased when the calcium was increased in the diet, and it was decreased by vitamin D The urinary calcium excretions continued to increase during Periods 10 and 11 and both the serum calcium and inorganic phosphorus values rose

Periods 12, 13, 14 and 15 differed from the previous two in that vitamin D was discontinued There was little reversal of trends until the last period Then the fecal calcium excretion rose sharply, the urinary calcium excretion fell, the fecal phosphorus excretion rose and the urinary phosphate showed no significant change

The above observations are quite clear cut. When the *modus operandi* of vitamin D is understood, it is probable that the cause of each change will be apparent There are many theories as to the action of vitamin D and obviously it will take many experiments to prove any one Before leaving the above data, however, it seems of interest to see which of the possible hypotheses these data support

When vitamin D was administered in sufficient doses in this patient there resulted six metabolic sequelae decrease of fecal calcium, moderate decrease of fecal phosphorus increase of urinary calcium, no increase of urinary phosphate, elevation of serum calcium, elevation of serum inorganic phosphorus In a previous experiment (1), furthermore, with the largest doses of vitamin D there was apparently a definite fall in the

urinary phosphorus excretion The opposite of these changes occurred when vitamin D was stopped If one assumes these five changes to be interrelated phenomena dependent on one fundamental change, the data are consistent with the hypothesis that vitamin D is primarily concerned with calcium (not phosphorus) and increases the absorption of calcium Thus the sequence after vitamin D administration would be (1) increased absorption of calcium causing decreased fecal calcium excretion, (2) with increased absorption of calcium an increase in urinary calcium excretion (cf Periods 3, 4, and 5 where increased absorption of calcium due to increased intake caused increased urinary calcium excretion), (3) with decreased calcium in feces, an increased phosphorus absorption and hence decreased fecal phosphorus excretion (cf rise in fecal phosphorus values in Periods 3 4 and 5 when calcium was given by mouth) (4) with increased calcium absorption an increased deposition of the calcium-phosphate-carbonate compounds dahlite, into the bones so that in spite of the increased phosphate absorption there is no increased urinary phosphate excretion

So much for the four excretory changes Before discussing the blood changes it might be well to point out that if one starts with an increased phosphate absorption as the primary action of vitamin D one meets with difficulties Thus this would lead to a decreased fecal phosphorus excretion, but an increased absorption should not be followed by a decreased urinary excretion Furthermore, since giving phosphates by mouth instead of intravenously had no effect on the fecal calcium excretions, it is hard to see how an increased absorption of phosphate in this patient could have affected the fecal calcium excretions The increased urinary calcium excretions would be difficult to explain since the giving of phosphate intravenously decreased the urinary calcium excretions Note, furthermore, that the fecal phosphorus excretions went from a maximum of 612 mgm before vitamin D therapy down to a minimum of 358 mgm with therapy The corresponding figures for calcium were 1157 mgm and 213 mgm

How is the elevation of serum inorganic phosphorus as a result of vitamin D therapy to be explained? The obvious explanation is that

increased absorption of both calcium and phosphorus there would be a tendency for both of these substances to rise in the serum There are certain difficulties, however The giving of large amounts of phosphate intravenously had no effect on the serum phosphorus level Furthermore, during Period 6 with a serum inorganic phosphorus level of 2 7 mgm he excreted 2465 mgm of phosphorus in the urine, in Period 13 with a blood value of 4 0 mgm he excreted 2376 mgm in the urine Thus as the blood phosphorus rose as the result of therapy, less phosphorus was excreted in the urine, or in any case there was no marked increase The findings can be explained if one brings the parathyroids into the picture

As pointed out in another paper (1) there is considerable evidence to suggest that the parathyroid glands are overactive in rickets This patient's parathyroid glands were found to be hyperplastic at operation and removal of one gland was followed by a marked rise in the serum inorganic phosphorus level and a fall in the serum calcium level This suggested that the preoperative values were kept where they were only by an excess of hormone production When part of this production was stopped by the operation, the remaining tissue, since it was already overactive, was unable to "take up the slack" Now, if one assumes that the stimulus for parathyroid hormone production is a low serum calcium level, then as vitamin D increases the calcium absorption and raises the serum calcium level, there would be less need for the parathyroid hormone With cessation of overactivity of the glands the serum phosphorus would rise This rise would not lead to an increased phosphate excretion in the urine, as the rising serum phosphorus level following parathyroidectomy is apparently the result of a decreased urinary phosphorus excretion (3) Thus, whereas the elevation of serum calcium is probably a result of increased absorption of calcium, the elevation of serum phosphorus may be very largely owing to a decreased level of parathyroid activity

Even at the expense of repetition, it may be useful to see how such an hypothesis concerning vitamin D fits the facts of rickets, the facts of vitamin D therapy, and the facts of vitamin D poisoning

With vitamin D lack, calcium is not absorbed, the blood calcium tends to fall, this tendency is immediately met by an increased excretion of parathyroid hormone, a low blood calcium being the stimulus for parathyroid hormone production, the parathyroid hormone lowers the blood phosphorus until dahlite is being absorbed rapidly enough from the bones to keep the blood calcium normal (see another paper (1) in which it was pointed out that along with the wide osteoid seams in rickets there are areas here and there showing very rapid bone absorption) With the normal blood calcium and low blood phosphate, it is impossible to deposit dahlite where bone formation is taking place and wide osteoid seams result With the increased fecal calcium excretion there results increased fecal phosphorus excretion With less phosphorus being absorbed, there will be less going out in the urine, and the ratio of urine phosphorous to fecal phosphorus will be low Inasmuch as the secondary hyperparathyroidism keeps the calcium normal and the phosphorus depressed, the bone changes will be produced especially readily with a low vitamin D, low phosphate diet, as any increase in the phosphate in the diet will lead to increased phosphate absorption, and it is the low serum inorganic phosphorus which is causing the bone changes Cases of rickets with a low serum calcium level as well as a low serum phosphorus level may represent examples where the parathyroid compensation mechanism has broken down With vitamin D therapy all these changes correct themselves

With vitamin D poisoning the calcium absorption is so rapid that the blood calcium rises above normal, this leads to a depression of parathyroid activity and a secondary hypoparathyroidism with a high blood inorganic phosphate level With both values high there is precipitation of calcium phosphate into the soft tissues and death The hypothesis without modification is inadequate in explaining the demineralization of the skeleton which occurs with massive doses of vitamin D The necessary modification will be discussed below

With this working hypothesis as to the action of vitamin D and its interrelationship with parathyroid activity in mind it will be of interest to examine the second investigation This was carried out on a boy with practically no parathy-

TABLE II
Metabolic data on Patient 2 with idiopathic hypoparathyroidism

Three-day period	Calcium				Phosphorus				Serum			Therapy
	Urine	Feces	In take	Bal ance	Urine	Feces	In take	Bal ance	Calcium	Phosphorus	Phosphatase	
	grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc.	mgm per 100 cc	Bodansky units	
1	0 07	0.26	0.30	−0 03	0 84	0 63	1 78	+0 31	4 8(I)*	12 3	8 9	Control period
2	0 07	0 17	0 30	+0 06	0 67	0.36	1 78	+0 75	5 1(III)	12 2	9 6	Control period
3	0 05	0 29	0.30	−0 04	0 60	0 69	1 78	+0 49				Control period
4	0 09	0.29	1 93	+1 55	0 39	0.54	1 78	+0 85	4 5(I)	8 0	12.3	Calcium gluconate i v
5	0 17	0 24	1 93	+1.52	0.35	0 42	1 78	+1 01	5.3(I)	9.2	12 2	Calcium gluconate i v
6	0 07	0 42	0.30	−0 19	0 50	0 79	1 78	+0 49	5 4(I)	6.5	13 4	Control period
7	0 07	0 48	0.30	−0.25	1 02	0 49	1 78	+0 27	4 5(II)	11 4		Control period
8	0 08	0.52	1 93	+1.33	1 02	0 54	1 78	+0 22				Calcium gluconate p o
9	0 06	1 70	1 93	+0 17	1 05	0 98	1 78	−0 25				Calcium gluconate p o
10	0 07	1 63	1 93	+0 23	1 17	0 89	1 78	−0 28	4.2(I)	12 1	6 4	Same plus vitamin D
11	0 06	1 23	1 93	+0 64	1 17	0 52	1 78	+0 09	5 8(II)	10 4	5 1	Same plus vitamin D
12	0 09	1 12	1 93	+0 72	1 26	0 40	1 78	+0 12	7 1(II)	9 5	8 6	Same plus vitamin D
13	0.34	0 87	1 93	+0 72	1 42	0.39	1 78	−0 03	9 1(III)	8.5	6 9	Same plus vitamin D

* Roman numeral indicates on which day of period determination was made.

roid tissue in all probability, so that the action of vitamin D can be divorced from secondary changes resulting from variations in parathyroid activity

Metabolic data on Patient 2 (M G H Number 4636) with idiopathic hypoparathyroidism

During the first three control three-day periods (Table II) the patient was on a low calcium, moderately low phosphorus diet Findings typical of idiopathic hypoparathyroidism were present, namely a high serum inorganic phosphorus level (12.3 mgm per 100 cc.), a low serum calcium level (4 8 mgm per 100 cc.), and a very low urinary calcium excretion

During Periods 4 and 5 he received 1 63 grams of calcium intravenously each period in the form of calcium gluconate.[1] With each injection of calcium there occurred only a transient immediate rise in the serum calcium level, the preinjection level being reached again in 12 hours There was

[1] 271 mgm. of calcium were given twice daily in 250 cc. of saline.

probably a tendency, however, for the serum calcium to rise over a period of days (cf serum calcium = 4 5 at beginning and 5 4 mgm at end) There was a slight increase in the urinary calcium excretion but no definite change in the fecal calcium excretion There was, therefore, a very marked rise in the calcium balance The serum inorganic phosphorus was decreased, the urinary phosphorus excretion was markedly decreased, the fecal phosphorus excretion remained unaltered, and the phosphorus balance was markedly elevated There seems little question that the added calcium united with phosphate and disappeared somewhere—not in the feces possibly in the bones It should be pointed out that a patient with idiopathic hypoparathyroidism was chosen for the determination of whether calcium given intravenously would appear in the feces because, such a patient s serum calcium level being low there would be less loss of the injected calcium into the urine.

Periods 6 and 7 are control periods with the same régime as in Periods 1, 2 and 3

During Periods 8 and 9 the same amount of calcium gluconate as during Periods 4 and 5 was given, but this time by mouth   If one examines Period 9 after equilibrium had been established, one notices that most of this calcium appeared in the feces   From this it can be concluded that it was not absorbed, because from Periods 4 and 5 it was clear that had calcium reached the systemic blood system it would not have been excreted back into the feces   Inasmuch as the calcium was not absorbed there were no other changes except that the fecal phosphorus value was slightly increased as was to be expected   The serum calcium and inorganic phosphorus values on the morning following the last day of this regime were essentially the same as during the control period (4 2 and 12 1 mgm respectively)

The stage was now set for the addition of large amounts of vitamin D   If its effect is a result of increased absorption of calcium from the gastrointestinal tract, its administration in this case should have been followed by the same changes as occurred when calcium was given intravenously   Such in most respects was the case   During Periods 10, 11, 12, and 13 this patient received 6 cc of 2500 D viosterol daily (= 600,000 I U)   The fecal calcium excretion fell from 1 70 grams to 0 87 gram (Period 13)   The serum calcium showed a definite tendency to rise, especially in the second two periods of this regime   The urinary calcium did not rise significantly until Period 13 when some critical threshold point in the serum calcium was passed   The calcium balances were markedly increased   The fecal phosphorus excretion was decreased   The urinary phosphorus excretion, however, was increased instead of being decreased   The quantities involved were such that the increased urinary excretion could be explained by increased phosphorus absorption   The serum inorganic phosphorus level fell

These changes were very similar to the findings obtained when calcium was given intravenously and support the hypothesis that one of the main actions of vitamin D is to increase the absorption of calcium   There was one discrepancy, however   When calcium was given intravenously, the urinary phosphorus excretion fell about 300 mgm per period, under the influence of vitamin D, on the other hand, the urinary phosphorus excretion rose about 300 mgm (Period 13)   The

first suggestion as to the cause of this discrepancy is that the increased absorption of phosphorus which occurs with vitamin D not only might prevent the expected drop in the urinary phosphorus excretion resulting from the increased absorption of calcium, but even might lead to the observed rise   While these data do not absolutely refute this possibility the quantities involved suggest that the urinary phosphorus excretion is too high for such an explanation   Later studies (see Experiment III) demonstrate more conclusively that such an explanation is untenable

The fact that the serum phosphorus level fell is most significant   Under most situations the serum phosphorus rises when one gives vitamin D   If that rise is owing to an accompanying decreased activity of the parathyroid glands as hypothesized here, then in a parathyroidless patient it is clear why the rise does not occur   Furthermore, if the action of vitamin D on phosphorus metabolism were primarily on its absorption from the gut, one would expect the serum phosphorus to rise with vitamin D therapy even in a parathyroidless patient, the fact that it didn't supports the hypothesis that the vitamin acts primarily on calcium absorption

This second investigation supports the hypothesis suggested from the first, but brings up the question whether something more is not happening under vitamin D therapy in the phosphorus metabolism than can be explained on the basis of increased calcium absorption

Metabolic data on Patient 3 (M G H Number 14727) with idiopathic hypoparathyroidism

The subject of this investigation had idiopathic hypoparathyroidism of a severe degree like the patient in the previous investigation   The data (Table III) are included in this paper to throw further light on the question discussed above whether the increased urinary phosphorus excretion resulting from vitamin D administration can be explained on the basis of an increased absorption of phosphorus   The patient was on a low calcium moderately low phosphorus diet to which was added 5 grams of calcium gluconate by mouth daily in divided doses

During the three control periods (Periods 11, 12, and 13) the expected findings were present low urinary calcium excretion, high partition of

TABLE III

Metabolic data on Patient 3 with idiopathic hypoparathyroidism

Three-day period	Calcium				Phosphorus				Serum			Therapy
	Urine	Feces	Intake	Balance	Urine	Feces	Intake	Balance	Calcium	Phosphorus	Phosphatase	
	grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc.	mgm per 100 cc	Bodansky units	Vitamin D— U S P units
11	0 02	1 19	1 67	+0 46	0 60	0 54	1 78	+0 64				
12	0 02	0 73	1 67	+0 92	0 76	0 43	1 78	+0 59				
13	0 02	1 18	1 67	+0 47	0 74	0 43	1 78	+0 61	6.5(I)*	7 6		
14	0 01	1 01	1 67	+0 65	0 73	0 56	1 78	+0 49	6 4(I) 5 7(II)	8 7 8 9		200 000 200 000 200 000
15	0 02	0 85	1 67	+0 80	0 92	0 50	1 78	+0.36	5 6(II) 6 0(III)	8 4 8 4	9 0	200 000 200 000 200 000
16	0 02	0 47	1 67	+1 18	0 99	0 42	1 78	+0.37	6 8(II)	8 0	7 5	400 000 400 000 400 000
17	0 02	0 44	1 67	+1.21	1 06	0 50	1 78	+0 22	7 6(I) 8 1(II)	8 0 8 1	7 2 7 9	400 000 400 000
18	0 04	0.28	1 67	+1 35	1 08	0.30	1 78	+0 40	9.3(II)	7 6	7 5	
19	0 05	0 39	1 67	+1.23	1 07	0 43	1 78	+0.28	9 5(II)	6 6	6 1	
20	0 07	0 30	1 67	+1 30	1 08	0 32	1 78	+0.38	10 1(I)	6 9		
21	0 08	0 43	1 67	+1 16	0 91	0.27	1 78	+0 60	11 7(III)	6 2		

* Roman numeral indicates on which day of period blood determination was done.

phosphorus in the feces, low serum calcium (6 4 mgm per 100 cc) and high serum phosphorus (8 7 mgm per 100 cc.)

During vitamin D administration (200 000 to 400,000 units daily) in Periods 14, 15, 16, and 17, there was the expected decrease in the fecal calcium excretion (about 600 mgm), a slight rise in the serum calcium level (from 6 4 mgm per 100 cc. to 8 1 mgm. per 100 cc.), and no change in the urinary calcium excretion The fecal phosphorus excretion showed no decided change, whereas the urinary phosphorus excretion rose decidedly, about 300 mgm The serum phosphorus level fell slightly

During the four control periods following cessation of vitamin D administration (Periods 18, 19, 20 and 21), the same trends continued until the last period when the vitamin effect began to wear off During these periods the fecal phosphorus excretion did show a definite decrease

This experiment makes it quite clear that all the changes following vitamin D administration cannot be explained on the basis of an increased calcium absorption It is apparently necessary to hypothesize an increased urinary phosphorus excretion as well This latter effect may be masked in patients with intact parathyroids by a decrease in the function of the parathyroid glands (see above) which leads to a decreased urinary phosphorus excretion (cf data on patient with rickets) In the experiment which follows, this observation concerning phosphorus excretion is even more convincingly demonstrated

Metabolic data on Patient 4 (M G H Number 8568) with idiopathic hypoparathyroidism

This investigation was essentially a repetition of the previous one except that the calcium intake by mouth was very low (0.30 gram per three-day period), additional calcium being administered daily in the form of calcium gluconate intravenously The data are shown in T   IV   they

TABLE IV

Metabolic data on Patient 4 with idiopathic hypoparathyroidism

Three-day period	Calcium				Phosphorus				Serum			Therapy
	Urine	Feces	Intake	Balance	Urine	Feces	Intake	Balance	Calcium	Phosphorus	Phosphatase	
12	grams 0 26	grams 0 55	grams 0 85	grams +0 04	grams 0 75	grams 0 67	grams 1 78	grams +0 36	mgm per 100 cc	mgm per 100 cc	Bodansky units	Vitamin D— U S P units
13	0 24	0 56	0 85	+0 05	0 95	0 67	1 78	+0 16	7 2(II)*	6 2		
14	0 26	0 42	0 85	+0 17	1 12	0 56	1 78	+0 10				
15	0 30	0 42	0 85	+0 13	1 01	0 58	1 78	+0 19	7 3(III)	6 7		400,000 400,000 400,000
16	0 33	0 36	0 85	+0 16	1 59	0 45	1 78	−0 26	8 6(II)	6 4		400,000 400,000 400,000
17	0 46	0 29	0 85	+0 10	1 52	0 37	1 78	−0 11	8 8(I) 9 0(III)	6 2 5 9		400,000 160,000
18	0 68	0 21	0 85	−0 04	1 62	0 39	1 78	−0 23	9 3(II)	6 1		200,000 400,000 400,000
19	0 94	0 13	0 85	−0 22	1 80	0 31	1 78	−0 33	9 9(II)	6 1		400,000 400,000 400,000
20	1 16	0 18	0 85	−0 49	1 52	0 42	1 78	−0 16	10 3(I) 10 1(III)	5 3 5 2		
21	1 44	0 17	0 85	−0 76	1 37	0 34	1 78	+0 07	9 7(III)	5 2		
22	1 52	0 15	0 85	−0 82	1 78	0 34	1 78	−0 34	9 9(III)	4 9		

* Roman numerals indicate on which day of period blood determination was done.

substantiate previous observations In addition it should be noted that the urinary calcium excretion increased much more than the fecal calcium excretion was decreased, leading to a negative calcium balance This again emphasizes the fact that all the metabolic changes cannot be explained by an increased calcium absorption The mobilization of calcium in this instance is probably a sequela of the increased phosphorus excretion

DISCUSSION

The main points have been discussed during the presentation of the data Of the two hypothesized fundamental actions of vitamin D—to decrease fecal calcium excretion and to increase urinary phosphorus excretion—it should be noted that the former would tend to heal rickets, the latter would tend toward demineralization In all the experiments here reported, massive doses of vitamin D were employed As discussed above, demineralization can occur when too large doses of vitamin D are given, a phenomenon which would be difficult to explain if the only action of vitamin D were on the calcium absorption It, therefore, seems possible that the effect on calcium absorption (the antirachitic action) may predominate unless too large doses are administered when demineralization may occur due to the effect on phosphorus excretion

The next question is whether the effects of vitamin D on calcium absorption and urinary phosphorus excretion are two separate actions of the vitamin or whether one is the more fundamental and the other secondary to it That the phosphorus excretion cannot be the result of increased calcium absorption was demonstrated in the second experiment when it was shown that

the giving of calcium intravenously decreased the phosphorus excretion There remains, therefore, only the question whether the increased urinary excretion of phosphorus could lead to the decreased fecal calcium excretion The data at hand do not give any evidence on this point. However, the parathyroid hormone causes a marked urinary excretion of phosphorus without any marked effect on calcium absorption Furthermore, other data (4) suggest that A T 10 (dihydrotachysterol) has the same two fundamental actions as vitamin D, but to different degrees With A.T 10 the ratio of the phosphorus excretion property to the calcium absorption property seemed greater The fact that the degree of one phenomenon obtained in relation to that of the other varies with different drugs is evidence that one is not dependent on the other

Certain observations from the literature are related to the observations here reported Nicolaysen (5) found in rats that the ingestion of calcium had a marked effect in increasing the fecal phosphorus excretion, but that the ingestion of phosphorus had very little effect on the fecal calcium excretion He likewise found that the fecal phosphorus was not increased by the parenteral injection of phosphates In a later paper (6) this same author found that vitamin D had a marked primary action on the absorption of calcium from the gut, but no such effect on the absorption of phosphorus and that the changes

in phosphorus absorption could be explained by the changes in calcium absorption Hannon, Liu, Chu, Wang, Chen, and Chou (7), studying the effect of vitamin D in osteomalacia, found that $CaCl_2$ administered intravenously was retained This suggested that the high fecal calcium excretion in that condition was due to lack of calcium absorption and not to increased reexcretion They also found that vitamin D, when first given, affected the calcium balance out of proportion to the theoretical phosphorus balance This suggested to them that changes in the phosphorus metabolism were the result of preceding changes in the calcium metabolism Liu and coworkers (8) described two types of osteomalacia In the first type there was a normal serum phosphorus, a low serum calcium, tetany, cataracts, and less bone trouble, in the second type there was a normal serum calcium, a low serum phosphorus, no tetany, and more bone disease Vitamin D healed both types These findings are in agreement with the theories here presented if one assumes that in the first type a secondary hyperparathyroidism failed to occur Wilder, Higgins, and Sheard (9) believed the parathyroid hyperplasia in rickets and osteomalacia decreased the amount of bone disease. This conclusion is contrary to the theories here discussed

Below an attempt is made to present in a diagram the rather confusing interrelations discussed in the paper
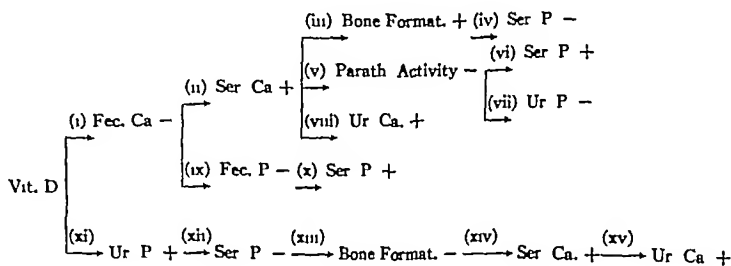
Whether the serum phosphorus level rises will depend on whether Arrows vi and x are greater than iv and xii, *etc*

On the basis of the serum calcium and phosphorus values three types of vitamin D deficiency may be hypothesized as occurring

(*a*) Low calcium, normal phosphorus parathyroid hyperplasia has not occurred

(*b*) Normal calcium, low parathyroid hyperplasia has calcium, and

(*c*) Low calcium, low

parathyroid hyperplasia has occurred but is unable to compensate for low calcium

SUMMARY AND CONCLUSIONS

1 Metabolic studies were performed on four patients Patient 1 was under treatment for a form of rickets very resistant but not intractable to vitamin D therapy, Patients 2, 3, and 4 had idiopathic hypoparathyroidism

2 An increase in the ingested calcium in Patient 1 was followed by an increase in the fecal phosphorus excretion, however, an increase in the phosphate ingested was not followed by an increase in the fecal calcium excretion These findings suggested that calcium in the diet has more influence on phosphate absorption than phosphate in the diet has on calcium absorption

3 The intravenous administration of large amounts of phosphates in Patient 1 was followed by no increase in the fecal excretion of phosphate, this suggested that reexcretion of phosphate into the gastro-intestinal tract was not influenced by the amount of phosphate entering the blood When the same amount of phosphate was given by mouth, there was likewise no increase in the fecal phosphorus excretion, this suggested that the amount of phosphate in the feces was independent of the amount of phosphate ingested Large amounts of vitamin D, however, decreased the fecal phosphorus excretion as well as the fecal calcium excretion, these findings suggested that the fecal phosphorus excretion depends on the fecal calcium excretion and that vitamin D decreased the latter

4 Vitamin D therapy in Patient 1, besides being followed by a decrease in the fecal excretions of calcium and phosphorus, led to an increased urinary calcium excretion, no increase in the urinary phosphorus excretion, and an elevation of both calcium and inorganic phosphorus in the serum The findings seemed consistent with the hypothesis that vitamin D increased the absorption of calcium, the other sequelae being secondary to this phenomenon The added proviso was necessary that with the rising serum calcium level there occurred a decreased activity of the parathyroid glands (see below)

5 The fact that with the administration of vitamin D the serum inorganic phosphorus value rose, whereas the urinary phosphorus excretion remained stationery or even fell, strongly suggested that the rise in the inorganic phosphorus level was owing to an accompanying decreased activity of the parathyroid glands as a result of therapy (cf decreased urinary phosphorus excretion and rising inorganic phosphorus level in serum with parathyroidectomy) That the rise in serum inorganic phosphorus level was not due merely to increased absorption of phosphorus was shown by its failure to occur when phosphate was administered intravenously

6 In Patient 2 the intravenous administration of calcium was followed by no increase in the calcium excretion in the feces, the same amount of calcium given by mouth caused a marked increase in the fecal calcium excretion Since vitamin D, thereafter, decreased the calcium excretion in the feces it was concluded that this was owing to increased absorption of calcium and not to decreased reexcretion into the gastro-intestinal tract

7 In Patients 2, 3, and 4 with probably no functioning parathyroid tissue, vitamin D therapy was followed by a falling serum inorganic phosphorus level This is further evidence that the rise in inorganic phosphorus which usually follows vitamin D therapy is the result of an accompanying decreased activity of the parathyroid glands

8 In Patients 2, 3, and 4, the administration of large amounts of vitamin D was followed by an increase in the urinary phosphorus excretion greater than could be explained by the decreased fecal phosphorus excretion This seemed to necessitate the added hypothesis that vitamin D in addition to increasing the absorption of calcium increases the urinary excretion of phosphorus It is probably because of this property of vitamin D that one gets demineralization with large doses

9 A tentative diagram is presented for the relation to one another of the various sequelae following vitamin D therapy

10 On the basis of the serum calcium and phosphorus values three types of vitamin D deficiency are differentiated (1) without parathyroid hyperplasia, (2) with compensatory hyperplasia, and (3) with hyperplasia, insufficient to cause compensation

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A COMPARISON OF THE EFFECTS OF A.T 10 (DIHYDROTACHYSTEROL) AND VITAMIN D ON CALCIUM AND PHOSPHORUS METABOLISM IN HYPOPARATHYROIDISM

By FULLER ALBRIGHT ESTHER BLOOMBERG TRUMAN DRAKE, and HIRSH W SULKOWITCH

(*From the Medical Service of the Massachusetts General Hospital and the Department of Medicine of the Harvard University Medical School Boston*)

Since the paper by Holtz in 1933 (1) there has appeared in the German literature a considerable number of articles on a photochemical derivative of ergosterin, designated dihydrotachysterol or A T 10 (antitetanisches Präparat Nr 10) Until recently this substance has not been available in this country One gets the impression from the German literature (see below) that A T 10 and vitamin D effect calcium metabolism in much the same way with the one extraordinary difference that A.T 10 is not antirachitic This combination of facts is most surprising since at first thought it is hard to believe that any substance, chemically so closely related to vitamin D, could have so similar an action on calcium metabolism and still not cure rickets It thus becomes of interest to know the exact manner in which this new substance effects calcium and phosphorous metabolism and wherein its action differs from that of vitamin D No complete metabolic data have as yet appeared Therefore the present investigations were undertaken They consist of calcium and phosphorus metabolic studies on 3 cases of hypoparathyroidism treated with A T 10, two of whom were later treated with vitamin D for comparison [1] Patients with hypoparathyroidism were chosen for this study so that secondary changes in calcium and phosphorus metabolism resulting from varying degrees of activity of the patient's own parathyroid tissue would not be confused with changes due to the A T 10 The importance of this aspect was discussed in a paper concerned with the action of vitamin D (2)

REVIEW OF LITERATURE

Bamburger (3) von Wendt (4) and Windaus (5) were the first to suggest that the toxic effect and anti

rachitic effect of irradiated ergosterin were owing to two different factors. The previous conception had been that the toxic manifestations—calcium deposits in various organs and hypercalcemia—were due to an overabundance of the antirachitic factor—hence, a hypervitaminosis. This toxic factor was called by Holtz *et al* (6) the " Calcinosefaktor " to differentiate it from the anti rachitic factor

Holtz Gissel and Rossmann (7) studied the various derivatives of ergosterin. It was found (7 8) that upon irradiation of ergosterin with ultraviolet light one obtained the following substances ergosterin, lumisterin, tachysterin vitamin D, toxisterin, suprasterin I and suprasterin II Of these tachysterin, vitamin D and toxisterin contained the " Calcinosefaktor " vitamin D alone, contained the antirachitic factor Holtz conceived the idea that the ' Calcinosefaktor " might be made use of in treating the hypocalcemia of hypoparathyroidism To this end tachysterin was converted chemically into dihydrotachysterol A.T 10, to render it suitable for peroral administration

A method of standardizing the toxic effect of ergosterin derivatives has been worked out (7) The unit of toxicity is designated the " Toxische Grenzdosis " (T G) and is that amount which given to mice for 12 days will cause a weight loss of at least 12 per cent. It is therefore possible to compare the antirachitic and the toxic properties of various preparations Harnapp gave the following figures (9)

1 cc vigantol [2]	4 500 antirachitic units	15 T G
1 cc A.T 10	400 antirachitic units	150 0 T G

Incidentally this is the only reference which suggests that A.T 10 is antirachitic at all

Furthermore, since the parathyroid hormone if given in excess likewise causes death to animals due to hypercalcemia and metastatic calcification it is possible to compare the effect of A.T 10 with that of parathyroid extract. Thus it was found that a dose of 0.2 to 10 unit of parathormone per kilo on thyroparathyroidectomized dogs was required to maintain a normal blood calcium level while it took 1.5 to 3.5 T G units of A.T 10 Hence 1 unit of parathormone corresponds to about

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[1] Part of the A T 10 and all of the vitamin D were supplied by Mr C. B Taft of the Winthrop Chemical Company The preparation of vitamin D used was " Drisdol " which is a preparation of crystalline vitamin D.

[2] Vigantol is the trade name for a preparation of irradiated ergosterol marketed by Merck of Darmstadt and by I G Farbenindustrie. One cc. vigantol 12,000 International Vitamin D units.

5 T G units dihydrotachysterol According to a recent symposium (10) A T 10 has been further purified and its biological activity is now assayed on parathyroidectomized dogs

Numerous papers state that A T 10 is not antirachitic and it seems that this fact must have been ascertained on animals The protocols of such experiments have not been found Harnapp (9), however, found that A T 10 had no effect in curing infantile rickets or in raising the serum calcium level in such cases Bomskov (10), on the other hand, found that A T 10 therapy will raise the serum calcium level in spasmophilia, but agrees that it will not correct the underlying rickets Eckert (10) found A T 10 effective in late rickets Dr Alfred Shohl at the Children's Hospital in Boston in some preliminary and as yet unpublished experiments found that A T 10 was not antirachitic in rats

The literature leaves no doubt that A T 10 is very effective for the treatment of hypoparathyroidism (11 to 31 inclusive) Reschke as quoted by Rieder (17) stated in 1934 that 200 cases had been treated without a single failure. Holtz (19) cited one patient who had been taking A T 10 for 3 years without any ill effect Another patient died of acute yellow atrophy after taking the drug for 2 years (340 cc. of A T 10 in all) and at autopsy showed no evidence of calcification of the kidneys It has also been used with benefit in tetany due to sprue (17, 18)

The dosage varies with the individual Holtz (19) states that 2 to 5 cc. of A T weekly should be sufficient in mild cases of tetany When the serum calcium has reached a normal level, the dosage is reduced Martini and Heymer (16) reported a case of postoperative tetany which required 10 cc. of A T 10 daily for about 2 weeks in order to obtain symptomatic relief Many observers warn against the dangers of overdosage which are increased by the marked cumulative action of the drug Arnold, Holtz, and Marx (32) found that 6 times as much A T 10 are required toward the end of pregnancy as otherwise. During periods of marked activity (8, 26), nervous strains, and menstruation a larger dosage is usually necessary The requirement for A T 10 decreases in women, after x-ray castration (29) The cataracts, so commonly seen in conjunction with hypoparathyroidism, can be kept from developing, but are not influenced by the drug once they have formed (10, 18, 19, 33)

The drug has been used with apparently good results in conditions other than those associated with hypocalcemia These include hemophilia (17, 18, 34, 35), impetigo herpetiformis (10), peripheral circulatory conditions (36), and a variety of other conditions Kappis as quoted by Rieder (18) treated "coeliakie." Gissel (35) reported good results using A T 10 in the treatment of bronchial asthma as well as in drug and serum eruptions Wendt and Altenburger (22) treated severe urticaria with the drug Danckelman (37) in 1934 reported treating painful varices and phlebitic residuals with A T 10

Very little data are available on the action of the drug on the body chemistry All observers agree that the serum calcium level is raised. Holtz and Kramer (38) found that both fractions of calcium are elevated, the ionized and that bound to protein In cases with tetany as opposed to normal controls the effect is more predominantly on the ionized fraction With elevation in the serum calcium level there is an increase in the urinary calcium excretion (41) which may lead to stone formation (38) The fecal calcium excretion is decreased (38, 39) Jordans (30) and Holtz and Kramer (38), furthermore, found that if a low calcium diet is used, calcium will be mobilized from the skeleton Hoff (40) in one case of hypoparathyroidism found a slight elevation in the serum phosphorus level after treatment followed by a later fall Snapper (14) and Arnold and Blum (26) found a lowering of the serum phosphorus level following treatment Otherwise no data have been found on the phosphorus metabolism Halbertsma (27) mentioned a mobilization of calcium with an increase in the calcium level of the blood, but with no increase in the serum phosphorus level Hoff (40) observed a slight rise in serum magnesium, no decided change in serum potassium, and a slight rise in the "alkali reserves" with A T 10 therapy Holtz (10) could not confirm the changes in the alkali reserve.

Arnold, Holtz, and Marx (32) found the action of A T 10 antagonized by estrin and the male sex hormone.

Experiment I

The subject, W C, Patient 3, Number 14,727, of the first investigation (2) was a 16 year old boy with severe idiopathic hypoparathyroidism A detailed clinical history will appear elsewhere (42)

The experiment was divided into Parts A and B In Part A he received A T 10, in Part B he received crystalline vitamin D Sufficient time was allowed to elapse between the two parts of the experiment so that his metabolic levels would return to the pretherapeutic values Throughout the entire experiment he received a diet of an exactly similar composition each day This consisted of a low calcium, moderately high phosphorus diet In addition, 0 45 of a gram of calcium in the form of calcium gluconate was given daily by mouth in three divided doses Thus, it was thought, sufficient calcium and phosphorus was being ingested to give the drug *something* to act upon if its action were on the absorption of calcium or phosphorus The data are shown in Figure 1 and Table IA and Table IB

During the three control periods (Periods 1, 2, and 3) there were present a low serum calcium level (7 0 mgm per 100 cc), a high serum phos-
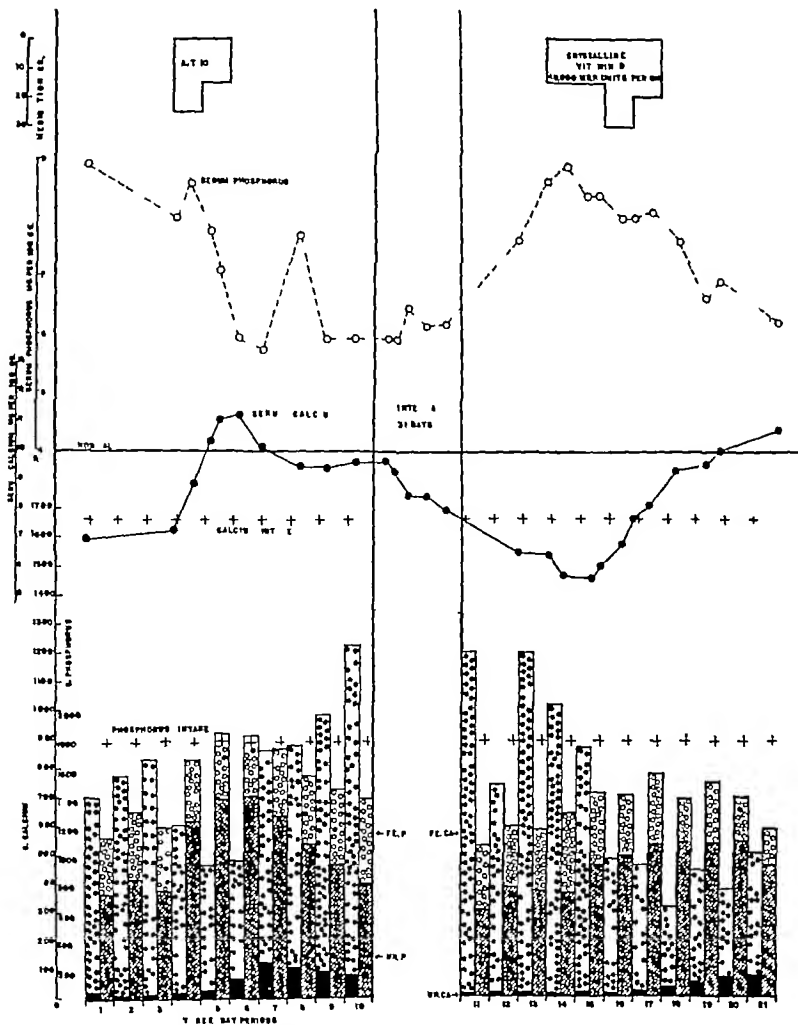
FIG 1   GRAPHIC REPRESENTATION OF METABOLIC DATE OF EXPERIMENT I

phorus level (9 0 mgm per 100 cc), the expected low urinary calcium excretion and a positive balance of both calcium and phosphorus

With the administration of A T 10 (Periods 4 and 5), there was an immediate decrease in the fecal calcium excretion but no change in the fecal phosphorus excretion   The urinary phosphorus excretion however, immediately rose, while the urinary calcium excretion remained unchanged The positive calcium balance was, therefore increased while the phosphorus balance was decreased and even became negative   The serum calcium level was elevated and the serum phosphorus level was depressed   With ъи

of the drug in Period 6, these effects continued unabated for that period only, in Period 7 the fecal calcium excretion again began to rise and the urinary phosphorus excretion began to fall The urinary calcium excretion did rise after the serum calcium level became normal and for some reason reached its highest level when the serum calcium was already falling

the experiment With the administration of vitamin D the fecal calcium excretion again decreases without any immediate change in the fecal phosphorus excretion, the urinary phosphorus excretion again rises without any immediate effect on the urinary calcium excretion The serum calcium level eventually rises and the serum phosphorus value eventually falls Finally the urin-

TABLE IA

Experiment I, Part A

Three-day period	Date	Calcium				Phosphorus				Serum			Therapy
		Urine	Feces	Intake	Balance	Urine	Feces	Intake	Balance	Calcium	Phosphorus	Phosphatase	
		grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc.	mgm per 100 cc	Bodansky units	
1	October 1936 8–9–10	0 03	0 68	1 67	+0 96	0 73	0 40	1 78	+0 65	7 0(I)*	9 0	7 0	
2	11–12–13	0 02	0 76	1 67	+0 89	0 83	0 47	1 78	+0 48				
3	14–15–16	0 02	0 81	1 67	+0 84	0 75	0 45	1 78	+0 58				
4	17–18–19	0 03	0 58	1 67	+1 06	1 24	0 43	1 78	+0 11	7 2(I) 8 9(III)	8 0 8 6	5 6	10 cc A T 10 10 cc. A T 10 5 cc A T 10
5	20–21–22	0 03	0 43	1 67	+1 21	1 39	0 45	1 78	−0 06	10 4(II) 11 1(III)	7 8 7 1	6 1	5 cc. A T 10 5 cc A T 10 5 cc A T 10
6	23–24–25	0 07	0 41	1 67	+1 19	1 41	0 42	1 78	−0 05	11 2(II)	5 9	4 1	
7	26–27–28	0 13	0 74	1 67	+0 80	1 27	0 47	1 78	+0 05	10 2(I)	5 7	4 3	
8	29–30–31	0 11	0 77	1 67	+0 79	1 07	0 47	1 78	+0 24	9 5(II)	7 7	2 6	
9	November 1–2–3	0 10	0 89	1 67	+0 68	0 92	0 52	1 78	+0 34	9 5(II)	5 9	4 5	
10	4–5–6	0 08	1 14	1 67	+0 45	0 80	0 59	1 78	+0 39	9 7(II)	5 9		

* Roman numerals indicate to which day of period data refer

From this experiment alone one would suspect that there are two primary metabolic effects of A T 10 (decrease of fecal calcium and increase of urinary phosphorus), that each of these lead to a secondary effect (rise in serum calcium and fall in serum phosphorus), and finally that one of these secondary effects (rise in serum calcium) in turn leads to a tertiary effect (increase in urinary calcium excretion)

When one turns to the second part of the experiment (Table IB), one finds the fecal calcium values in the control periods (Periods 11, 12, and 13) considerably higher than those in similar periods (Periods 1, 2, and 3) in the 'first half of

ary calcium excretion rises There is also a delayed fall in the fecal phosphorus excretion, suggesting that eventually the body compensates for the increased urinary phosphate excretion by an increased absorption of phosphorus

It will be noted that all the changes were in the same direction as when A T 10 was administered[3] There were, however, certain quantitative differences The effect of vitamin D in the

[3] This statement is not quite true as there was no delayed decrease of fecal phosphorus excretion when A T 10 was administered If one judges from later experiments, however (Tables IIA and III), this was because A T 10 was not administered for a sufficiently long time

doses used was slower in coming on, on the other hand, the effect, once there remained longer after discontinuance of treatment. Secondly, if one confines ones attention to the two primary effects (to decrease the fecal calcium and to increase the urinary phosphorus), it will be noted that vitamin D in the doses used effected fecal calcium as ef-

that of A T 10, whereas the effect of vitamin D on the urinary phosphorus excretion was decidedly less than that of A T 10

Experiment II

The subject D B , Number 8568, of the second investigation, was a girl of 21 with severe

TABLE IB
Experiment I Part B

Three-day period	Date	Calcium				Phosphorus				Serum			Therapy
		Urine	Feces	In take	Bal ance	Urine	Feces	In take	Bal ance	Calcium	Phos-phorus	Phos-phatase	
		grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc	mgm per 100 cc	Bodansky units	Vitamin D— U S P units
11	December 1936 8–9–10	0 02	1 19	1 67	+0 46	0 60	0 54	1 78	+0 64				
12	11–12–13	0 02	0 73	1 67	+0 92	0 76	0 43	1 78	+0 59				
13	14–15–16	0 02	1 18	1 67	+0 47	0 74	0 43	1 78	+0 61	6 5(I)*	7 6		
14	17–18–19	0 01	1 01	1 67	+0 65	0 73	0.56	1 78	+0 49	6 4(I) 5 7(II)	8 7 8 9		200 000 200 000 200,000
15	20–21–22	0 02	0 85	1 67	+0 80	0 92	0 50	1 78	+0.36	5 6(II) 6 0(III)	8 4 8 4	9 0	200 000 200 000 200 000
16	23–24–25	0 02	0 47	1 67	+1 18	0 99	0 42	1 78	+0.37	6 8(II)	8 0	7 5	400 000 400 000 400 000
17	26–27–28	0 02	0 44	1 67	+1 21	1 06	0 50	1 78	+0.22	7 6(I) 8 1(II)	8 0 8 1	7 2 7 9	400 000 400 000
18	29–30–31	0 04	0 28	1 67	+1 35	1 08	0 30	1 78	+0 40	9 3(II)	7 6	7 5	
19	January 1937 1–2–3	0 05	0.39	1 67	+1.23	1 07	0 43	1 78	+0.28	9 5(II)	6 6	6 1	
20	4–5–6	0 07	0.30	1 67	+1 30	1 08	0.32	1 78	+0 38	10 1(I)	6 9		
21	7–8–9	0 08	0 43	1 67	+1 16	0 91	0 27	1 78	+0 60	11 7(III)	6.2		

* Roman numerals indicate to which day of period data refer

fectively as A T 10 if not more so On the other hand, the action on the phosphorus excretion in the urine was less marked with vitamin D than with A T 10

The observations in the second part of this experiment could be summarized, therefore, by saying that the primary, secondary, and tertiary effects of vitamin D were qualitatively the same as those of A T 10 However, the effect of vitamin D was slower in coming on and lasted longer Finally, the effect of vitamin D on the fecal calcium excretion was as great if not greater than

idiopathic hypoparathyroidism A detailed clinical history will appear elsewhere (42)

Since in Experiment I it had been shown that A T 10 could raise the serum calcium value by decreasing the fecal calcium excretion, it seemed of interest to try to determine whether the serum calcium could be raised on a low calcium intake In other words, was A T 10 only effecting calcium absorption or could it prevent calcium excretion into the gut or even mobilize skeletal calcium? Unfortunately, because of the patient's severe degree of tetany it was impossible to carry
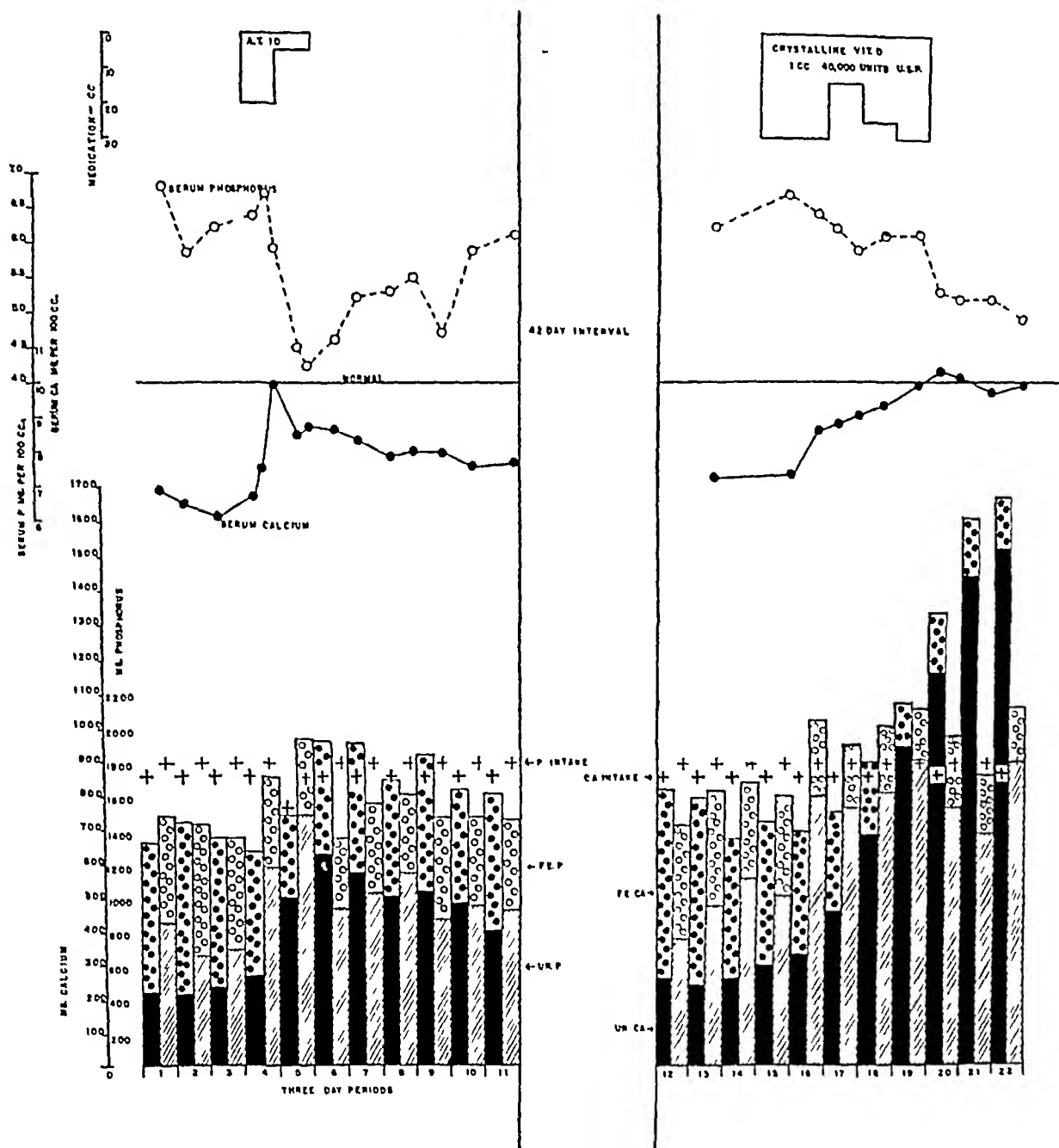
FIG 2 GRAPHIC REPRESENTATION OF METABOLIC DATA OF EXPERIMENT II

her for control periods on a low calcium diet without added medication Therefore, it was decided to put her on a low calcium diet (0 10 gram per day) and to give her intravenously, in addition, calcium gluconate (0 18 gram of calcium per day) in two divided doses All blood samples were taken at least twelve hours after the previous intravenous calcium administration

The experiment was divided into Parts A and

B During Part A she received A T 10 and during Part B she received crystalline vitamin D The diet was of an exactly similar composition throughout Sufficient time was allowed to elapse between Parts A and B to let the equilibria return to the premedication levels The data are shown in Figure 2 and Tables IIA and IIB

During the first three control periods, 1, 2, and 3, she had much more calcium in her urine

than she would have had with a serum calcium value of 6.9 mgm., had she not been receiving intravenous calcium medication The excess (about 180 mgm) undoubtedly resulted from the calcium given intravenously It is of further interest that *slightly more calcium was excreted in*

fecal phosphorus excretion, although not nearly large enough to account for the increased urinary phosphorus excretion

There was a rise in urinary calcium excretion greater than the fall in fecal calcium excretion so that the calcium balance was decreased and even

TABLE IIA

Experiment II Part A

Three-day period	Date	Calcium				Phosphorus				Serum		Therapy
		Urine	Feces	Intake	Balance	Urine	Feces	Intake	Balance	Calcium	Phosphorus	
		grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc.	mgm per 100 cc	
1	February 1937 2–3–4	0 21	0 45	0 85*	+0 19	0 85	0 61	1 78	+0 32	6 9(II)†	6 8	
2	5–6–7	0 21	0 51	0 85	+0 13	0 65	0 77	1 78	+0 36	6 5(I)	5 8	
3	8–9–10	0.23	0 44	0 85	+0 18	0 69	0 66	1 78	+0 43	6 1(I)	6.2	
4	11–12–13	0 27	0.37	0 85	+0.21	1 18	0.53	1 78	+0 07	6 7(I) 7 5(II) 9 9(III)	6 4 6 7 5 9	10 cc. A.T 10 5 cc. A.T 10 5 cc. A.T 10
5	14–15–16	0 50	0 24	0 76	+0 02	1 48	0 44	1 78	−0 14	8.5(II) 8 7(III)	4 5 4.3	5 cc. A T 10
6	17–18–19	0 63	0 33	0 85	−0 11	0 93	0 42	1 78	+0 43	8 6(II)	4 6	
7	20–21–22	0 57	0 37	0 85	−0 09	1 02	0 52	1 78	+0.24	8.3(I)	5 2	
8	23–24–25	0 50	0.34	0 85	+0 01	1 14	0 46	1 78	+0 18	7 9(I) 8 0(III)	5 3 5.5	
9	26–27–28	0.52	0 40	0 85	−0 07	0 88	0.59	1 78	+0 31	8 0(II)	4 7	
10	March 1–2–3	0 48	0.33	0 85	+0 04	0 95	0.52	1 78	+0 31	7 6(II)	5 9	
11	4–5–6	0 40	0 40	0 85	+0 05	0 93	0 52	1 78	+0.33	7 7(III)	6 1	

* Patient received 10 cc. of 10 per cent calcium gluconate intravenously twice daily throughout the entire experiment except for Period 5 during which one injection was omitted Therefore, the ingested calcium was 0.31 gram and the ingested calcium 0.54 gram
† Roman numerals indicate to which day of period data refer

the feces than was given by mouth This confirms the work of Nicolayson (43) that the fecal calcium does not merely represent unabsorbed calcium in the diet

During the periods of A T 10 administration (Periods 4 and 5) the fecal calcium excretion was decreased as in the previous experiment but not by more than the calcium in the diet

Again, as in Experiment I, A T 10 had a very marked effect (about 100 per cent) on the urinary phosphorus excretion with the production of a negative phosphorus balance In this experiment, however, there was a slight decrease in the

became negative during Periods 6 and 7 This demonstrates that calcium can be mobilized from the skeleton with A T 10

In Part B it is seen that again the effect of vitamin D was qualitatively the same as that of A.T 10 The data again suggest that the action of vitamin D is slower and lasts longer On the whole, it seems fair to conclude also that the ratio of the action of A T 10 on the urinary phosphorus excretion to that of its action on calcium absorption was greater with A.T 10 than with vitamin D In comparing Parts A and B in this respect, allowance must be made for the fact that

TABLE IIB

Experiment II, Part B

Three-day period	Date	Calcium				Phosphorus				Serum		Therapy
		Urine	Feces	Intake	Balance	Urine	Feces	Intake	Balance	Calcium	Phosphorus	
		grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc	mgm per 100 cc	Vitamin D— U S P units
12	April 1937 18–19–20	0 26	0 55	0 85	+0 04	0 75	0 67	1 78	+0 36			
13	21–22–23	0 24	0 56	0 85	+0 05	0 95	0 67	1 78	+0 16	7 2(II)*	6 2	
14	24–25–26	0 26	0 42	0 85	+0 17	1 12	0 56	1 78	+0 10			
15	27–28–29	0 30	0 42	0 85	+0 13	1 01	0 58	1 78	+0 19	7 3(III)	6 7	400,000 400,000 400,000
16	May 30–1–2	0 33	0 36	0 85	+0 16	1 59	0 45	1 78	−0 26	8 6(II)	6 4	400,000 400,000 400,000
17	3–4–5	0 46	0 29	0 85	+0 10	1 52	0 37	1 78	−0 11	8 8(I) 9 0(III)	6 2 5 9	400,000 160,000
18	6–7–8	0 68	0 21	0 85	−0 04	1 62	0 39	1 78	−0 23	9 3(II)	6 1	200,000 400,000 400,000
19	9–10–11	0 94	0 13	0 85	−0 22	1 80	0 31	1 78	−0 33	9 9(II)	6 1	400,000 400,000 400,000
20	12–13–14	1 16	0 18	0 85	−0 49	1 52	0 42	1 78	−0 16	10 3(I) 10 1(III)	5 3 5 2	
21	15–16–17	1 44	0 17	0 85	−0 76	1 37	0 34	1 78	+0 07	9 7(III)	5 2	
22	18–19–20	1 52	0 15	0 85	−0 82	1 78	0 34	1 78	−0 34	9 9(III)	4 9	

* Roman numerals indicate to which day of period data refer

TABLE III

Metabolic data on Experiment III

Three-day period	Date	Calcium				Phosphorus				Serum		Therapy
		Urine	Feces	Intake	Balance	Urine	Feces	Intake	Balance	Calcium	Phosphorus	
		grams	grams	grams	grams	grams	grams	grams	grams	mgm per 100 cc	mgm per 100 cc	
1	May 1937 29–30–31	0 08	0 82	1 67	+0 77	0 75	0 50	1 78	+0 53			
2	June 1–2–3	0 11	1 03	1 67	+0 53	0 94	0 70	1 78	+0 14			
3	4–5–6	0 08	1 20	1 67	+0 39	1 55	0 57	1 78	−0 34	5 7(I)*	8 6	5 cc A T 10 5 cc A T 10 5 cc A T 10
4	7–8–9	0 19	0 81	1 67	+0 67	1 75	0 40	1 78	−0 37	7 1(I) 7 9(III)	7 6 6 3	5 cc A T 10 5 cc. A T 10
5	10–11–12	0 33	0 77	1 67	+0 57	1 18	0 35	1 78	+0 26	8 1(II)	6 1	1 cc A T 10

* Roman numerals indicate to which day of period data refer

vitamin D therapy was pushed much further than that with A.T 10 Furthermore, since there was very little calcium to absorb (cf low calcium diet) there was a limit to the amount by which the drugs could decrease the fecal calcium excretion

The patient received a diet exactly similar to that used in Experiment I During the control periods (Periods 1 and 2) the expected findings were present although the urinary calcium excretion was slightly higher than one would expect
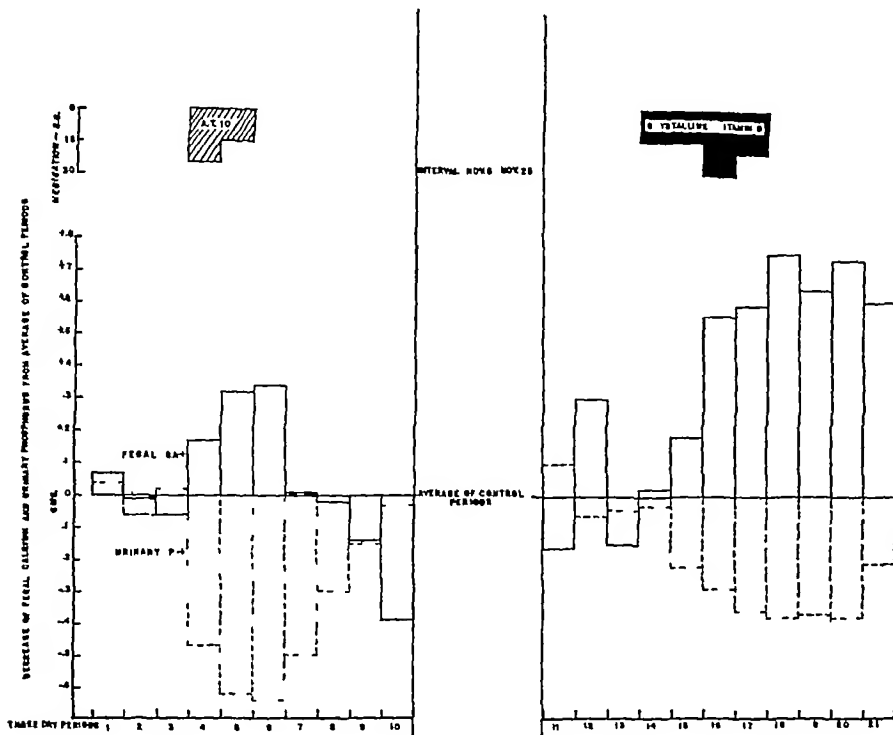
FIG 3

This chart is constructed from data of Experiment I Tables IA and IB It is designed to show that the ratio of decrease in fecal Ca excretion to increase in urinary P excretion is greater with vitamin D than with A.T 10 Base line represents average fecal Ca excretion and average urinary P excretion of control periods. The difference between the average values and the values for each period are plotted for each period—above the line if the difference represents a saving to the body, below the line if a loss It will be noted that the Ca effect is greater with vitamin D while the P effect is greater with A T 10

Experiment III

The subject, M F McC , Number 48512 of this experiment was a woman of 40 with severe post-operative hypoparathyroidism, for which she had been followed at the Massachusetts General Hospital since March, 1930 The thyroid operation had been performed at another hospital in June, 1929

The blood values before medication were for the serum calcium 5 7 and for the serum phosphorus 8 6 mgm per 100 cc (Table III)

The patient received A.T 10 during Periods 3, 4, and 5 The most striking effect again was on the urinary phosphorus excretion, there being about a 100 per cent increase over the control periods during Period 4 The

TABLE IV

Data showing effect of parathyroid hormone in hypoparathyroidism

Three-day period	Calcium				Phosphorus				Serum		Therapy
	Urine	Feces	Intake	Balance	Urine	Feces	Intake	Balance	Calcium	Phosphorus	
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*mgm per 100 cc*	*mgm per 100 cc*	*parathormone ** units*
20	0 12	2 60	3 85	+1 13	0 58	0 70	1 99	+0 71	8 9(I)*	6 9	
21	0 11	2 40	3 85	+1 34	0 54	0 65	1 99	+0 80	8 9(II)	6 9	
22	0 11	2 94	3 85	+0 80	0 50	0 96	1 99	+0 53	8 6(II)	7 3	
23	0 12	2 71	3 85	+1 02	0 45	0 62	1 99	+0 92	8 8(III)	6 8	
24	0 18	3 33	3 85	+0 34	1 62	0 81	1 99	−0 44	8 3(I) 10 5(II) 11 0(III)	7 3 5 0 5 0	40 40 40
25	0 27	2 24	3 85	+1 34	0 97	0 77	1 99	+0 25	10 2(II)	5 4	40 40 40
26	0 33	2 47	3 85	+1 05	1 13	0 49	1 99	+0 37	12 3(I) 11 2(II)	5 3 4 7	40 60 60

* Roman numerals indicate to which day of period data refer
** One unit of parathormone is equivalent to 5 units of parathyroid extract

value fell from 8 6 mgm to 6 1 mgm There was a slight decrease in the fecal calcium excretion and a slight rise in the urinary calcium excretion The net result was that the calcium balance was little affected while the phosphorus balance was made negative These findings strengthen the observations made in the previous experiments

DISCUSSION

In a previous paper (2) the tentative hypothesis was made that there are two fundamental actions of vitamin D—to increase calcium absorption and to increase urinary phosphorus excretion A T 10 has both of these properties, but the ratio of the effect on phosphorus excretion to that on calcium absorption is apparently greater with A T 10 than with vitamin D (Figure 3) Since the calcium absorption effect is obviously an antirachitic one whereas the phosphate excretion effect would work in the opposite direction, some explanation is afforded as to why A T 10 is not antirachitic

Since one of the main actions of A T 10 appears to be to increase the excretion of urinary phosphorus and since the theory has been advanced that this is the fundamental action of the parathyroid hormone (44) it becomes of interest to compare the metabolic action of A T 10 with that of the parathyroid hormone A priori one would expect them to be the same except for the fact that A T 10 has an additional action in causing increased calcium absorption

In Table IV, data are given on the effect of the parathyroid hormone on the calcium and phosphorus metabolism of the same individual with postoperative hypoparathyroidism studied in Experiment III During the control periods (Periods 20, 21, 22, and 23), the patient had a positive balance of both calcium and phosphorus, the serum calcium was 8 9 mgm per 100 cc and the serum phosphorus was 6 9 mgm per 100 cc Up to the onset of the experiment the patient had been receiving large amounts of vitamin D which explains why the serum calcium was as high as it was (Table III)

With administration of parathormone in Period 24 there was the expected large increase in the urinary excretion of phosphorus with a lowering of the serum phosphorus The fecal calcium ex-

cretion was little, if at all, influenced, however With a lowering of the serum phosphorus there was a rise in the serum calcium and an increased

an additional action on calcium metabolism which the parathyroid hormone does not possess (Figure 4)
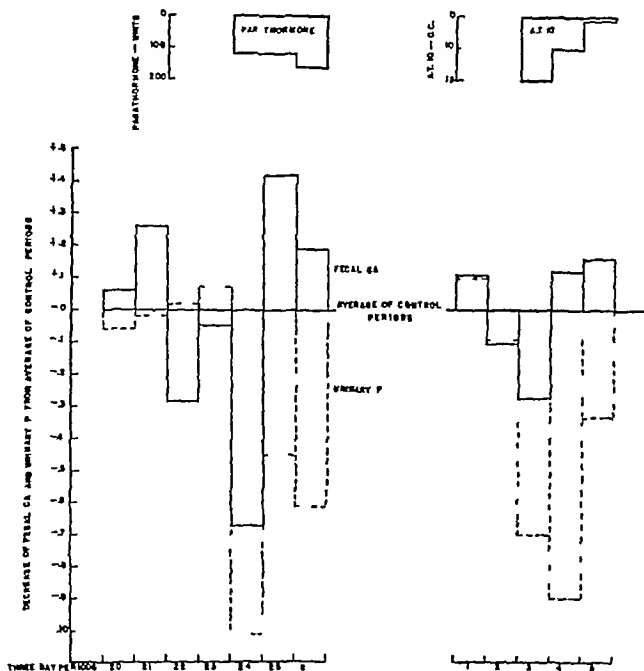
FIG. 4

This is constructed similarly to Figure 3 (q v) It is designed to show the difference between the effect of the parathyroid hormone on fecal Ca and urinary P excretions and that of A.T 10.

The chart is constructed from the data in Tables III and IV It will be noted that both A.T 10 and the parathyroid hormone have a similar effect on urinary P excretion. The fecal calcium excretion in the para thormone experiment however showed no constant variations. The large fluctuations were to be expected because of the large Ca intake in that experiment. A T 10 on the other hand probably did have a definite effect on fecal calcium (although one would not be sure from this experiment above)

calcium excretion in the urine. The findings fit the hypothesis that the fundamental action of the parathyroid hormone on phosphorus metabolism is the same as that of A T 10 while A.T 10 has

In a previous paper (2) the following diagr was presented to show the main actions of vita D on calcium and phosphorus metabolism and sequelae of these actions

```
                                      (iii) Bone Format  +      (iv) Ser P  −
                                     ┌──────────            ┌──────────→
                      (ii) Ser Ca +  │  (v) Parath  Activity − │  (vi) Ser P +
                     ┌──────→        │                        ┌──→
       (i) Fec Ca −  │               │  (viii) Ur Ca +         │  (vii) Ur P −
      ┌──────→       │                                        └──────→
Vit D │              │  (ix) Fec P −    (x) Ser P +
      │              └──────→          ──────→
      │
      └(xi) Ur P +   (xii) Ser P −    (xiii) Bone Format −   (xiv) Ser Ca + (xv) Ur Ca +
        ──────→       ──────→                                ──────→
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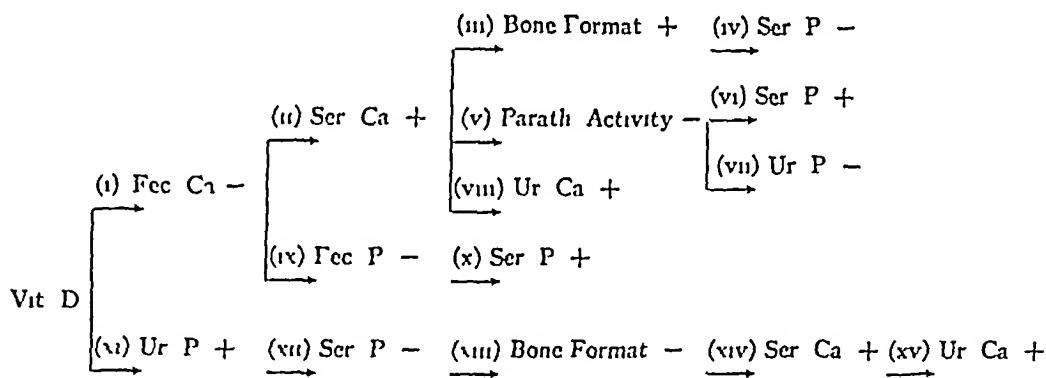
A similar diagram for A T 10 would be the same except that Arrow xi and its sequelae would be more accentuated In a diagram for the parathyroid hormone Arrow xi would be accentuated, likewise, whereas Arrow i and all its sequelae would be absent [4]

The studies here reported, therefore, suggest that the biological action of A T 10 differs from that of vitamin D in the direction of the parathyroid hormone It would seem that if the difference could be somewhat further extended, one might actually synthesize a substance with the same biological properties as the parathyroid hormone, if not the hormone itself If that were the case the parathyroid hormone would join that large group of hormones with chemical compositions resembling cholesterol

SUMMARY AND CONCLUSIONS

1 The actions of A T 10 and of vitamin D on the calcium and phosphorus metabolism of three patients with hypoparathyroidism were studied, in one of the three patients the effect of the parathyroid hormone was likewise determined

2 Both vitamin D and A T 10 had the same fundamental two actions—to increase calcium absorption from the gut and to increase phosphorus excretion in the urine, the ratio of the latter action to the former, however, was apparently greater with A T 10, which may explain why A T 10 is not antirachitic

3 The action of vitamin D was slower in coming on and lasted longer than that of A T 10

4 The parathyroid hormone resembled A T

10 as regards its property of causing a markedly increased urinary phosphorus excretion but differed in that it probably had no primary action on calcium absorption from the gut

5 Because of the similarity between the actions of the parathyroid hormone and of A T 10, the latter drug is a most efficacious therapeutic agent in the treatment of hypoparathyroidism

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[4] These diagrams are presented, not that the authors have much hope that they will not have to be modified at a later date, but to help correlate a rather confusing mass of data

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EVAPORATION OF BODY WATER IN LOBAR PNEUMONIA [1]

By GEORGE K. ANDERSON

(From the Departments of Physiology and Pediatrics University of Rochester School of Medicine and Dentistry Rochester N Y)

In order to maintain a constant body temperature the heat loss must equal the heat production The modes of heat loss are by radiation, conduction, convection, and water evaporation This last varies greatly according to the bodily require ments Certain modifications in the rate of evaporation, concomitant with fever, are the subject of discussion in this paper

The non renal loss of water, often called insensible loss, consists of water evaporated from the skin and from the respiratory passages Benedict and Root (1) showed that the total evaporation rate parallels the oxygen consumption so closely that under limited conditions it serves as a measure of the energy metabolism of the body Benedict and Root determined that the loss from the lungs among a variety of human individuals averages 35 per cent of the total insensible water loss in the resting individual

Since the insensible perspiration so closely parallels the oxygen consumption in health, it has been of interest to determine any changes induced during fever Leyden (2) was one of the earliest investigators to demonstrate by daily determinations of body weight, a retention of water during fever The first measurements in pneumonia of evaporation from the body surface, exclusive of the head, were recorded by Schwenkenbecher (3) From a patient with lobar pneumonia he found less evaporation at the temperature of 39.9° C than on the morning after the crisis when the temperature was 37 6° C Other investigators recorded a greater rate of water loss during certain stages of fever than in the normal state

Sandelowsky (4) reported seven cases of lobar pneumonia in which there was a decrease in body weight appearing concurrently with the disappearance of the fever A concentration of the blood was found along with the decreased body weight Sandelowsky considers this as evidence of rapid

loss of water from the blood and body tissues At the same time this author reports three cases out of ten which show no such change.

Lussky and Friedstein (5) associated a loss of body weight at the time of defervescence in twenty nine cases of lobar pneumonia in infants with a water retention during the febrile period

Sunderman and Austin (6) made careful observations on the water balance in six cases of lobar pneumonia They concluded that there is a negative balance of water in most cases of lobar pneumonia under routine care, and either a decrease in negative, or else a positive, water balance when pneumonia patients are given plentiful NaCl in their diets

Ribadeau-Dumas and Meyer (7) reported determinations of water evaporation in cases of bronchopneumonia in infants Weighing the child for one-half or one hour periods on a balance sensitive to 1 gram, they find that in most cases the perspiration is increased 40 to 60 per cent over normal, with resulting weight loss, while in two cases considerably diminished perspiration and some edema are found

EXPERIMENTAL PROCEDURE

The subjects of these observations were children in the Pediatric Divisions of the Strong Memorial and Rochester Municipal Hospitals under routine care for lobar pneumonia, on diets consisting chiefly of fluids during the febrile period The children were carried from their beds into a room having a slow circulation of air from a conditioning unit within the insulated room with a constant temperature of 28° C and approximately 35 per cent relative humidity The children usually wore no more than a pair of trunks and were placed upon a rubber sheet on the Sauter balance. This balance is sensitive to 0 1 gram and has a double-swing period of approximately 30 seconds The time required for the loss of 2 grams of weight in small children and 5 grams in older children, taken in one or sometimes suc-

cessive periods, determined the water evaporation in grams per hour   After entering the room, a period of 5 to 10 minutes elapsed before the weighing was made while the child became relaxed and accustomed to his surroundings

When a separation of insensible loss into loss from skin and loss from lungs was desired, the subject was made to breathe through flutter valves to a bottle of 4 to 8 mesh pumice stone saturated with concentrated H_2SO_4 This bottle rode upon the patient's side of the balance and collected the water of the expired air   The difference in weight before and after collection of respiratory water vapor was then determined

Actual body weight was determined at the time of evaporation measurements   So far as possible, the weighings were done at the same hour each day to exclude the effects of excitement, baths, or recent meals   Since the patients were available for study only while in the hospital, the stay in some cases was too short for complete return to a healthy state and for comparison with the febrile period

RESULTS

Seven cases of lobar pneumonia in children were studied   In two of these, partition of the lung and skin evaporation was made   The results in these seven uncomplicated cases show that the vaporization of water ran typical courses, one of which is represented graphically in Figure 1
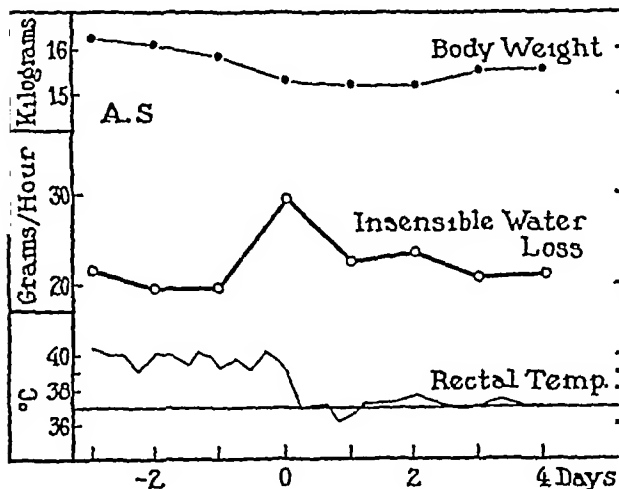
FIG 1   PATIENT A S   DAILY RELATION OF INSENSIBLE WATER LOSS TO BODY WEIGHT AND RECTAL TEMPERATURE IN LOBAR PNEUMONIA

The patients were first studied in the febrile state   Comparison of the rate of water evaporation at this time with that determined in the late recovery period showed an increase in body evaporation during the fever in five cases, no change in one, and a slightly lower value in another   The average departure from normal (late recovery period) was a 10 per cent increase

Since the patients were not available during the development of the fever, the changes in evaporation rate which might be observed at that time may be inferred from the values found during the subsequent fall of the temperature, and from measurements made upon isolated instances of rising temperature as found in two cases of bronchopneumonia and one case with a daily spiking fever of unknown etiology   These unrecorded observations and the changes to be subsequently described which are found during the abatement of fever all indicate a definite decrease in rate of evaporation during the development of fever

If, during fever, this body temperature remains constant, the rate of evaporation varies but little, but with either a rise or a fall of rectal temperature, the vaporization rate is markedly altered showing a rise during a drop in temperature.   At the time of the crisis, as the temperature starts to fall, the insensible perspiration becomes sensible perspiration   This change seen as the peak upon the chart, usually reaches its maximum within the 12 hours following the onset of defervescence, although in two cases it was not recorded until the second day following the crisis   This may be attributable to the infrequency of measurements   The average increased rate of water loss during the crisis as compared to that in the late recovery period is $+47$ per cent   Following this critical period, the rate of evaporation gradually returns to the basal value over a period of 3 to 4 days

On the charts are shown the daily determinations of body weight   With the children partaking of fluids almost exclusively, and thus having a low caloric intake, the highest value appears just before the subsidence of the fever (best shown in Figure 2)   Just after the crisis with its heightened vaporization, the body weight shows a sudden decrease and does not recover until two or more days after the crisis   It would seem as
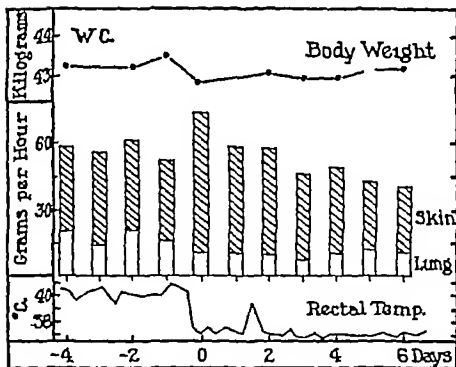
FIG 2. PATIENT W C. DAILY RELATION OF SKIN AND LUNG FRACTIONS OF WATER LOSS TO BODY WEIGHT AND RECTAL TEMPERATURE IN LOBAR PNEUMONIA.
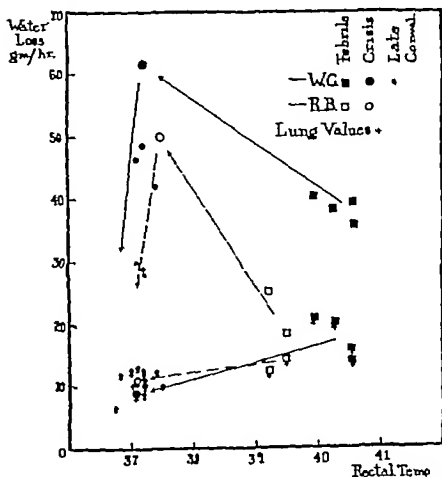
FIG 3 RELATION OF WATER LOST THROUGH SKIN (ABOVE) AND WATER LOST FROM LUNGS (BELOW) TO THE RECTAL TEMPERATURES IN TWO CASES OF LOBAR PNEUMONIA.

Arrows indicate sequences of successive measurements

if the sudden body weight loss and the body water loss were two measurements of the same thing

In two cases, simultaneous determinations of skin and lung water loss were made Case W C in Figure 2 shows the greatest febrile increase in total insensible water loss being an increase of 26 per cent over normal The fraction lost from the skin is decreased relative to increased total

skin and lung loss at this time. The highest values for the lung evaporation are recorded during the fever state, giving a skin to lung ratio of 2 1 During the crisis, the skin loss increases greatly and the lung evaporation decreases to make a ratio of 6 1 Subsequently the skin loss decreases over 3 to 4 days until the nearly constant value is reached The lung loss remains quite constant through convalescence, the last measurements showing a skin to lung ratio of 3 1

TABLE I

Insensible water loss on successive days, divided into three arbitrary periods in seven cases of lobar pneumonia.
(Numbers represent grams per square meter per hour)

Patient	Age	Surface area sq. meters	Fever				Crisis				Late convalescence		
			Day −4	Day −3	Day −2	Day −1	0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	years												
W C.	8	1.26	46.5	45.0	43.5	41.1	57 4	46.5	46.0	36.1	38.7	33.4	31 1
R. B	13	1.19			23.0	37 5	40.1			30.2	23.8	25.7	25.2
R. B	10	1 12			20.1	29 1	50.2*		32.2		21.9	22.5	
A. C.	5	0.76				30.9	43.5	50 1	53.1	46.4	41.6	37 7	34.2
R. R.	9	0.98			37.0	34.1	46.7*	39 1	35.0	33.3	37.2		35.4
A S.	6	0 71		30.4	23.6	23.6	42.1	32.0	33.1	29.2	29.6		
J D	14	1.48		25.4	32.4	25.0	35.1	46.5*	39.2		39.6		34.5
Means				34.0	32.5	32.4	45.2	42 8	40.5	35.0	33.6	29.1	30.1
Means of daily means					33.1			44.0				30.1	

* The highest value measured, usually at time of crisis
† This figure is not included in the averages.

DISCUSSION

The rate of water evaporation cannot be used as a measure of body metabolism in lobar pneumonia. One investigator reports an increase of as much as 50 per cent in oxygen consumption during lobar pneumonia (8) while the water evaporation studies presented here show an average increase of 10 per cent during the fever There is obviously a shift in heat loss by water vaporization, for water is not eliminated in proportion to water intake (6) nor to the rate of energy metabolism The main source of upset in water loss is seen in the diminished skin evaporation This decreased evaporation may be a factor in the rise of body temperature which in turn produces a rise in energy metabolism One explanation for this decreased evaporation from the skin may be found in the peripheral vasoconstriction noted in human capillaries by Fremont-Smith et al (9) at the onset of fever The chill

so often experienced at the onset of febrile lobar pneumonia in humans may be owing to this cutaneous vasoconstriction

While a large component of the heat and water losses, the water evaporation from the skin, is underfunctioning, the loss from the lungs becomes increasingly efficient This is due to the elevated body temperature and hence greater vapor tension of the expired air, and to the increased ventilation afforded by the rapid shallow respirations of lobar pneumonia (10) The increased respiratory water loss with rapid shallow respirations is described by Christie and Loomis (11) They suggest that this type of respiration excessively ventilates those parts of the lungs below the bronchi and above the alveoli to produce " a water loss out of proportion to the CO_2 loss "

While the heat loss by water evaporation is deficient during fever, that share of the heat lost by radiation, conduction, and convection is increased and may be said to be proportional to the body temperature Measurements made by the author by taking two or three consecutive thermocouple readings from several skin areas show an average rise of one degree skin temperature for each degree rise in rectal temperature in lobar pneumonia

A balance is made by the elevated energy metabolism and faulty skin evaporation on one hand and the increased skin radiation and respiratory vaporization on the other, such that the body temperature levels off at the persistent high value of the fever state

At the time of the crisis, the temperature falls rapidly This is accomplished by a rapid vaporization of water from the skin, it is only by this means that the heat can be lost as rapidly as it is An opinion might be expressed that in lobar pneumonia the changes in skin evaporation appear to allow a more rapid abatement than development of the high fever

SUMMARY

1 An increase in the body weight during the fever of lobar pneumonia in children is found

2 The insensible water loss through the skin as measured on a Sauter balance is less than normal in amount during the rise of the fever (determined in fevers other than lobar pneumonia), closely approximates normal skin values during the maintained fever, and is markedly elevated at the time of the crisis and gradually decreases for 3 to 4 days thereafter

3 The lung vaporization is highest during the febrile period, taking over in part the deficient heat loss of the skin at this time

4 The average skin temperature from several areas shows one degree rise for each degree rise in rectal temperature

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OBSERVATIONS ON THE ETIOLOGIC RELATIONSHIP OF ACHYLIA GASTRICA TO PERNICIOUS ANEMIA. VII RESEMBLANCES BETWEEN THE PROTEOLYTIC ACTIVITY OF NORMAL HUMAN GASTRIC JUICE ON CASEIN IN NEUTRAL SOLUTION AND THE ACTIVITY OF THE INTRINSIC FACTOR [1]

By F H L. TAYLOR, W B CASTLE, ROBERT W HEINLE AND MARGARET A ADAMS

(From the Thorndike Memorial Laboratory Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine Harvard Medical School Boston)

A CORRELATION OF THE *in vitro* ACTIVITY OF GASTRIC JUICE ON CASEIN AT pH 7 4 WITH THE CLINICAL ACTIVITY OF THE GASTRIC INTRINSIC FACTOR

It has been shown (1, 2) that the oral administration of normal human gastric juice (intrinsic factor) together with beef muscle (extrinsic factor) causes increased blood production and clinical improvement in patients with addisonian pernicious anemia Positive effects appear if a mixture of beef muscle and gastric juice, with or without preliminary incubation, is administered to the patient at pH 5 or 7 (2) Since neither beef muscle (1) nor gastric juice (2 3, 4 5, 6, 7) when administered alone has a positive effect on blood production, it has been inferred that an interaction between these substances is [hace for] hematopoiesis (2, 3, 7) Such clinito th[es]e, however, does not serve to indicate fact[or th]is interaction occurs *in vitro*, within gastr[o]ary tract, or parenterally

anem[a ve]ry recently, no evidence has been obto the[a]ny chemical activity *in vitro* in neutral Th[se o]f beef muscle and gastric juice. Un lub[e]experiments in 1930, in collaboraClif[f]Dr C W Heath, on mixtures of beef dai[ly a]d gastric juice incubated *in vitro* at pH nic[he]d in no detectable increases in the amino gast[ric]content of the mixture. Klein and inc[e]'s (8) claim that they have demonm[the] synthesis of the thermostable prin con[se]ver by *in vitro* interaction of beef mus fai[l]intrinsic factor from hog s gastric mu fact[no]ot been sustained in our hands when envi[ro]

mixtures of beef muscle and gastric juice were so employed (9) In 1934, however, Griffiths (10) reported increases in total nitrogen in trichloracetic acid filtrates of digests of normal human gastric juice incubated at pH 6 with beef muscle globulin He differentiated this activity from that of trypsin and pepsin only on the basis of the reaction of the mixture. Because of the smaller amounts of nitrogen freed by the secretions of patients with pernicious anemia, he pointed out the possibility of identity of this activity of normal human gastric juice with that of the so-called gastric intrinsic factor Emerson and Helmer (11) subsequently attributed the proteolysis described by Griffiths to a combination of slight peptic activity at pH 6 and differential adsorption of nonprotein substances by the proteins of the digest.

The results of recent clinical observations (9), however, suggest that some essential interaction between beef muscle and normal gastric juice does occur within the alimentary tract of the patient with pernicious anemia When a mixture of 200 grams of beef muscle and 150 ml of normal gastric juice was incubated for 6 hours at pH 1 8 or 2 5 and was given daily at pH 1 8 or 2.5 to patients with pernicious anemia, increased blood production failed to occur, but when the mixture was given at pH 5 or 7 following such acid incubation for 6 or for 12 hours, increased blood production did appear Thus, incubation in this acid medium for 12 hours apparently did not destroy intrinsic factor Instead it appears probable that the acid reaction of the mixture, maintained after administration to the patient by the buffering properties of the beef muscle protein, failed to provide the more nearly neutral environ ment suitable for the essential interaction of the beef muscle and gastric juice Since the

to produce from casein solution, upon incubation at pH 7 4, progressive increases in nitrogenous bodies not precipitable by trichloracetic acid The fact that the gastric juice employed, which was visually, at least, free from bile, did not contain significant amounts of regurgitated duodenal secretion (trypsin or erepsin) is confirmed by the relatively slight production of amino nitrogen (Table II, Experiments 19 and 35a, Table III, Experiment 15a, Table VI, Experiments 65a,

TABLE VI

Negative effect of incubation with equal quantity of 1 per cent casein solution at 37 5° C and pH 7 4 of gastric juice after treatment with Lloyd's reagent

Experiment number	Method of preparation of gastric juice	Increase in nitrogen in trichloracetic acid filtrates		Increase in amino nitrogen by formol titration	
		(mgm per 100 ml digest)			
		4 hours	24 hours	4 hours	24 hours
67b	Lloyd's reagent at pH 1 8 (18)	0 0	0 0	0 0	0 0
70b		0 0	0 0	0 0	0 0
65b	Lloyd's reagent at pH 7 4	0 04	0 3	0 0	0 0
70c		0 1	1 1	0 0	0 0
68b	Lloyd's reagent at pH 7 4 after incubation at pH 10 for 2 hours	0 0	0 2	0 0	0 0
67a	Control—normal gastric juice	3 9	15 5	0 0	0 0
70a		2 8	12 3	0 0	0 0
65a		52 5	53 9	1 0	2 0
68a	Control—normal gastric juice after incubation at pH 10 for 2 hours	27 9	47 2	0 0	0 0

67a, and 70a) despite considerable increases in total filtrable nitrogen Although saliva was undoubtedly present in the samples of gastric juice employed, this secretion alone was shown to be incapable of significant activity on casein at pH 7 4 (Table IV, Experiment 35d) It thus appears reasonable to conclude that the active agent in the samples of normal human gastric contents was secreted by the stomach

The use of washed casein (which is not vitamin free) as a substrate does not necessarily imply that as such casein is clinically an effective extrinsic factor That the activity observed was in fact due to the presence of the so-called intrinsic factor has been established only so far as the

correspondences between the *in vitro* observations presented above and the present clinical knowledge of the characteristics of the intrinsic factor allow such an inference Since, according to Helmer and Fouts, only one-half to two-thirds of the intrinsic factor was removed by Lloyd's reagent, the total removal of the *in vitro* activity by this reagent in our hands needs further study It is possible, however, that differences in the content of mucus or of other components of the gastric juice utilized, by interfering with the activity of Lloyd's reagent, may explain the differences in the results obtained

Since our preliminary report (12), Dr Fritz Lasch (19) of Vienna has published the results of independent observations on the activity of pepsin-free gastric secretion on powdered beef muscle and other substrates at pH 5 5 to 6 The gastric secretion of a variety of patients without anemia had significantly greater activity in producing nitrogen in trichloracetic acid filtrates of such digests than did samples of such gastric secretion after boiling Moreover, filtrates from digests containing gastric juice from several patients with pernicious anemia showed little or no increases in nitrogen In patients with hypochromic anemia and achylia and in patients with achylia without pernicious anemia the ability of the gastric secretion to produce nonprotein nitrogen agrees with certain clinical observations (20), demonstrating the presence of intrinsic factor Thus, further significant correspondences between *in vitro* and clinical observations on intrinsic factor have been established by Lasch

In connection with work demonstrating that the substance responsible for the blood forming activity of liver upon administration in pernicious anemia is probably an "albumose," Dakin, Ungley and West (21) have recently drawn attention to the work of Glaessner, who in 1902 described proteolytic activity in extracts of the gastric (22) and the duodenal (23) mucosa of the hog This activity he ascribed to an enzyme which he called "pseudo pepsin" Glaessner found it active on protein in weakly acid or alkaline solution, and stated that the cleavage products contained tryptophane Glaessner's work was partially confirmed by Reach (24) and Pekelharing (25) On the other hand, the existence of pseudo pepsin was denied by Klug (26), while Bergman (27) iden-

tified it with erepsin Subsequently, the finding of trypsin in gastric juice on occasion seemed to explain the proteolytic activity ascribed to pseudo pepsin Since at that time there appeared to be no known physiological need for such an enzyme, further attempts to prove or disprove its existence were not made In retrospect, however, the observations of Glaessner now appear to possess a renewed interest It also now seems probable that Griffiths (10) was correct in reporting proteolytic activity of human gastric juice at pH 6, despite the fact that his work could not be confirmed by Emerson and Helmer (11) Finally, since little or no amino nitrogen is produced from casein by the action of the gastric juice, the reason for our failure in 1930 to detect increases in the amino nitrogen content of digests of neutral mixtures of beef muscle and gastric juice now becomes obvious

B THE NATURE OF THE *in vitro* ACTIVITY OF NORMAL HUMAN GASTRIC JUICE ON CASEIN AT pH 7 4

The foregoing observations strongly suggest that some factor in normal human gastric juice when incubated with casein solution at pH 7 4 causes a progressive increase in the amount of nitrogen in the digest, which cannot be precipitated by trichloracetic acid It is possible that because of the correspondence of such activity with the characteristics of the intrinsic factor, as determined by clinical observation, the same agent may be active in each instance Certain other explanations must however, be considered It is possible that the increase in nonprecipitable nitrogen may have been due to (1) peptic hydrolysis, (2) differences in adsorption upon the protein substrate of soluble nitrogenous substances in different types or preparations of gastric juice (11), or (3) the action of tryptic or ereptic like enzymes Finally it is to be emphasized that since only small increases in amino nitrogen have been observed, the finding of changes in the amount of nitrogenous material in the digest not precipitated by trichloracetic acid, as observed by Griffiths (10), Lasch (19) and ourselves (12) does not necessarily constitute a demonstration of proteolytic activity Accordingly, with respect to our experiments, these possibilities were examined

Exclusion of pepsin

Since it is generally conceded that pepsin possesses little activity at pH ranges greater than its isoelectric point (pH 4 7), this enzyme could scarcely have been responsible for the considerable activity observed in our digests at pH 7 4 Further proof of this is afforded by the negative results of incubation of a 2.5 per cent solution of pepsin with casein at pH 7 4, as shown in Table IV, Experiments 30 and 32a Moreover, since pepsin is readily destroyed by alkali the fact that exposure of the gastric juice to pH 10 for 30 minutes did not significantly affect its subsequent activity at pH 7 4 (Table II, Experiments 33a and 35c) would appear to exclude pepsin Again, after exposure of gastric juice to pH 10 for 2 hours, its activity at pH 2 5, as judged by the Mett's tube method or by incubation with casein, was completely destroyed (Table V, Experiment 42b), though its activity at pH 7 4 on casein was retained (Table V, Experiment 42a)

Exclusion of trypsin and crepsin

Since samples of normal human gastric juice were discarded if visibly tinged with bile and since the increase in amino nitrogen from casein was trivial compared with the increase in total filtrable nitrogen (Table II, Experiments 19 and 35a Table III, Experiment 15a and Table VI Experiments 65a, 67a, and 70a), significant contamination with duodenal contents would seem to have been excluded However, the possibility that the activity demonstrated may have been due to a tryptic or ereptic-like enzyme of gastric origin needs consideration, especially as this supposition formed the basis of some of the objections (26, 27) to Glaessner's work The fact that Northrop (17) has shown that between 70 and 80 per cent of trypsin in solution is destroyed by exposure to alkali at pH 10 for 30 minutes at 40° C seemed to offer a method of discrimination This procedure was therefore applied respectively to normal gastric juice, to normal gastric juice purposely contaminated with duodenal contents and to gastric juice from two patients with pernicious anemia obviously containing regurgitated duodenal contents

Preliminary exposure of gastric juice to alkali did not significantly affect the increases in nitrogen

sembles in its time relations that produced by pepsin, the fact that the subsequent activity of the gastric juice was *not significantly affected* by exposure to alkali and that thereafter activity was maximal at about pH 8 and absent at pH 2 5 would appear *to exclude pepsin acting in its usual manner* Pepsinogen should have been entirely converted to pepsin by the natural acidity of the gastric juice and would then have been destroyed by the alkali If any pepsinogen had remained unconverted to pepsin, it should have become active as pepsin when the gastric juice was brought to pH 2 5 It thus appears that a type of proteolysis resembling that of pepsin in acid solution occurs when normal human gastric juice acts upon casein in a neutral environment Whether or not there is any relationship between the enzyme reported by Glaessner in 1902 and the proteolytic activity of gastric juice described in *this communication remains to be determined*

CONCLUSIONS

1 Incubation at 37 5° C and pH 7 4 of equal quantities of normal human gastric juice and 1 per cent casein solution results in progressive increases in the nitrogenous substances in trichloracetic filtrates of such digests

2 This activity of normal human gastric secretion, like that of the so-called intrinsic factor, is apparently (*a*) independent of the presence of saliva and of regurgitated duodenal contents, (*b*) absent or greatly diminished in the gastric secretion of patients with addisonian pernicious anemia, according to our observations and those of Lasch, (*c*) not destroyed by Berkefeld filtration or exposure to alkali, destroyed by exposure to 40° C for 72 hours, or to 70° to 80° C for 30 minutes, or by boiling for 5 minutes, and (*d*) inhibited by an environment more acid than pH 3 5

3 The *in vitro* activity of normal human gastric juice is entirely removed by treatment with Lloyd's reagent, which, however, Helmer and Fouts have shown by clinical test to effect only partial removal of the intrinsic factor

4 As determined by a modification of the nitrogen partition method of Wasteneys and Borsook, hydrolysis of casein by gastric juice at 37 5° C and pH 7 4 progresses within 24 hours chiefly to the stage of proteoses and peptones,

with production of relatively little amino nitrogen

5 This evidence is regarded as consistent with the action of a proteolytic enzyme

6 The proteolysis observed is considered not to be due to pepsin acting in its accepted manner because the activity was not *significantly affected* by exposure to alkali and thereafter was maximal at about pH 8 and absent at pH 2 5

7 The proteolysis observed is considered *not* to be due to tryptic or ereptic-like enzymes acting in their accepted manner because the activity was not significantly affected by exposure to alkali, because thereafter significant activity was observed at both pH 5 and pH 7 4, and because relatively little amino nitrogen was produced within 24 hours at 37 5° C

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THE EXCRETION OF UREA IN NORMAL MAN AND IN SUBJECTS WITH GLOMERULONEPHRITIS [1]

By HERBERT CHASIS and HOMER W SMITH

(From the Departments of Medicine and Physiology New York University College of Medicine New York City)

It is well known that the magnitude of the urea clearance in man is related to the rate of water excretion (urine flow), this relationship being commonly expressed in terms of the "standard" and "maximum" clearances of Möller, McIntosh and Van Slyke (20) But the actual physiological basis for the relationship is undetermined It is recognized that the rate of excretion of urea in the higher animals is considerably less than would be expected on the basis of glomerular filtration this was first indicated by Mayrs (19) from a comparison of the rates of excretion of various substances in the rabbit, and later substantiated for this animal by the investigations of MacKay and Cockrill (17), for man by Rehberg (25), for the dog by Jolliffe and Smith (16), the seal by Smith (32), the sheep by Shannon (27), and the chicken by Pitts and Korr (23)

Rehberg (25) and Holten and Rehberg (13) suggested that the relationship between urea clearance and urine flow in man is owing to the passive diffusion of urea across the tubules in consequence of the creation of a concentration gradient by the reabsorption of water from the glomerular filtrate Van Slyke, Rhoads, Hiller, and Alving (37) and Gordon, Alving, Kretzschmar, and Alpert (11) have observed in dogs with explanted kidneys that, although over any considerable period of time the urea clearance bears a fairly constant relationship to the rate of filtration, the fraction reabsorbed may vary markedly from moment to moment, but the reasons for this variation are unknown.

In the absence of any substantial evidence to the contrary, it may be assumed that the deficit between the urea clearance and the filtration rate in man is due entirely to tubular reabsorption, but in view of Shannon's (27) observations on the dog it is clear that this reabsorption is not related in a simple manner to the rate of water excretion

No systematic observations have been recorded on the relationship of the urea clearance to the rate of glomerular filtration and of water excretion in man Numerous investigators have examined the simultaneous creatinine and urea clearances in normal man and in subjects with acute or chronic diffuse glomerulonephritis, among whom may be mentioned Rehberg (24, 25), Holten and Rehberg (13, 14), Cope (6), Hayman, Halsted, and Seyler (12), Ellis and Weiss (8, 9,), Cambier (5), Covian and Rehberg (7), Winkler and Parra (38 39), Bjering (3) and Bing and Bjering (2) The most important facts established by these investigations are that the urea clearance (with few questionable exceptions) is less than the creatinine clearance, and that in renal disease the two clearances are reduced in a roughly parallel manner A few instances have been recorded in which the urea clearance was excessively reduced, relative to the creatinine clearance, indicating, as Rehberg (25, 13) suggests increased permeability of the tubules and abnormal back diffusion of urea

In view of the evidence against the acceptance of the creatinine clearance as a measure of glomerular filtration in man (26, 33), in view of the importance of the possibility of increased permeability of the tubules in glomerulonephritis, and in view of the inadequacy of our knowledge concerning the mechanism of excretion of urea, it was felt that a study of simultaneous inulin and urea clearances in normal subjects and in subjects with renal disease would be valuable.

The present report includes observations on 10 subjects with normal renal function and 22 sub-

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jects with acute or chronic glomerulonephritis [2] All subjects were patients on the Medical Service of the Third (New York University) Division of Bellevue Hospital The normal subjects had been admitted for treatment of diseases other than those of the genito-urinary or cardiovascular system They gave no history of renal disease and at the time of examination they had apparently normal renal function After convalescence they volunteered to act as subjects for this study They were under the supervision of a nurse engaged in an investigative capacity and they were given a mixed diet containing approximately 225 grams of carbohydrate, 110 grams of fat and 70 grams of protein, with an adequate supply of minerals and vitamins They received no drug therapy during the course of observation Three normal subjects were chosen for a special examination of the urea clearance in relation to the rate of urine excretion In each series of observations on these subjects, clearance determinations were made over as wide a range of urine flow as possible The subject was hydrated by the administration of 3 liters of water daily for 1 to 3 days previous to observation, on the morning of observation he received no breakfast but drank 2 liters of water, the last water being administered at least 45 minutes before the first clearance period Since it was impossible to obtain low urine flows after diuresis, these clearances had to be determined at essentially constant urine flows during moderate dehydration effected by abstinence from water for 48 to 60 hours In general, each series of observations continued over a period of two and one-half hours, the urine collection periods varying according to the rate of urine flow from 7 to 25 minutes The general methods of examination of all subjects, the administration of inulin by constant intravenous infusion, the collection of urine and methods of chemical analysis have been described by Smith, Goldring, and Chasis (34)

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[2] A preliminary report has been made on the inulin and phenol red clearance, etc in these nephritic subjects by Goldring and Smith (10) and a full report will be made later This report constitutes a full discussion of the excretion of urea in these subjects, and we are indebted to Dr Goldring for the opportunity to present these observations

NORMAL SUBJECTS

Effect of inulin on urea clearance

In order to determine whether the administration of inulin has any effect on the urea clearance, we have examined the latter in one subject (C B) in 34 periods without, and 39 periods with, simultaneous inulin clearances These observations will not be reported in detail, but it may be said that when the urea clearance is plotted against urine flow the two sets of data, with and without inulin, are entirely superimposable It is concluded that neither inulin nor our method of administering it by constant intravenous infusion changes the specific relationship of the urea clearance to the rate of urine flow

Relation between filtration rate and urine flow

We present for examination of this question 130 simultaneous inulin and urea clearances in 3 normal subjects (E B , T G , and C B) who were the object of special study, and 95 simultaneous inulin and urea clearances in 7 other normal subjects,[3] the latter being examined when the rate of urine formation was falling or had attained a moderately steady rate after water diuresis The average inulin clearance for the individuals in this group, excluding Subject C B ,[4] ranged from 110 0 to 128 8, with a mean of 120 5, compared to the mean normal value of 122 5 cc per 1 73 sq m per minute (34)

There is a slight correlation between the rate of filtration and the urine flow, as will be seen in the detailed data on Subject E B given in Figure 1, in the data on Subject T G which have been recorded by Smith (Figure 6 (33)) and in the mass data on all subjects given in Figure 2 It is admittedly difficult to interpret this correlation, since several incidental factors may in-

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[3] These 7 subjects are mentioned in the summary presented in Table III of Smith, Goldring and Chasis (34)

[4] Subject C B although giving no history of renal disease, showed average urea and inulin clearances of about 50 and 95 cc respectively, these values lie outside the range of normal values, as observed by Smith, Goldring and Chasis (34) He also showed anomalous renal function in that it was impossible to raise his urine flow by extreme water diuresis above 8 0 cc per minute. But in respect to the urea/inulin clearance ratio he behaved like the other normal subjects examined here, and data upon him are included in Figure 4
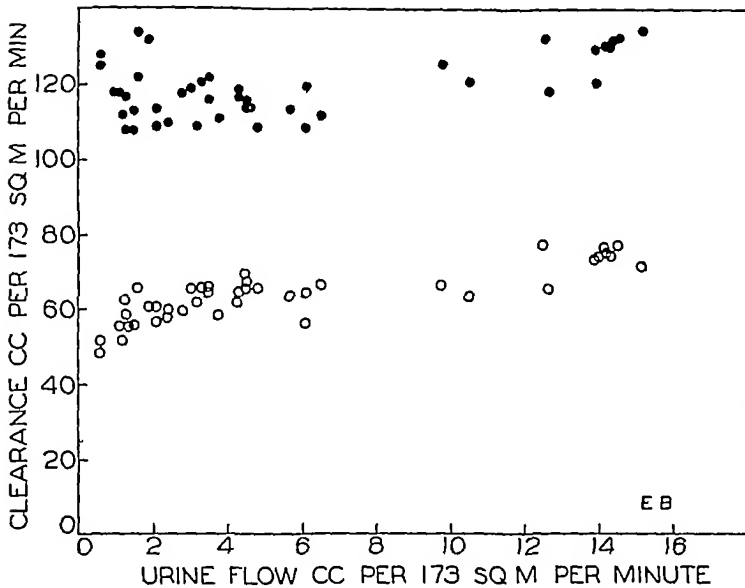
FIG. 1   42 SIMULTANEOUS INULIN (*dots*) AND UREA (*circles*) CLEARANCES ON SUBJECT E. B DURING FALLING OR CONSTANT URINE FLOWS

Each datum is a single clearance period.

fluence the filtration rate. For example, we are inclined to attribute the evident tendency for the filtration rate to increase above the average value at high urine flows to hydremia, increased circulation, etc., attending the administration of large quantities of water and the tendency for the filtration rate to fall below the average value at low urine flows to extrarenal factors such as anhydremia, oligemia decreased cardiac output or vasomotor effects of dehydration We recognize that when the filtration rate can, under basal conditions, vary in a single individual by ± 10 cc per minute, it would be hazardous to conclude that slight changes in this process have no physiological relationship to urine flow but in the same subject at the same filtration rate the urine flow may vary on different occasions from 1 0 to 16 cc. per minute, or at the same urine flow the filtration rate may vary from 110 to 130 cc. Such correlation as may exist at normal filtration rates appears to be incidental and to have no physiological relation to urine flow within the range of

0 5 to 20 cc per minute   This conclusion is in agreement with the lack of correlation between the creatinine clearance and urine flow, as demonstrated by Holten and Rehberg (13), Covian and Rehberg (7) and others   (We have not examined the filtration rate at urine flows below 0 5 cc per minute since such low flows cannot be obtained without excessive dehydration, particularly when saline is being given by constant intravenous infusion)

Possible conversion of urea to ammonia

Since urea is completely filtrable from the plasma the difference between the urea clearance and the filtration rate must be owing to removal of urea from the glomerular filtrate. The possibility that the deficit in the urea clearance in acidosis is a result of conversion of filtered urea to ammonia has been disproved in the dog by Pitts (22) and Alving and Gordon (1) though whether this is true for man is uncertain (36) That such conversion does not contribute to the
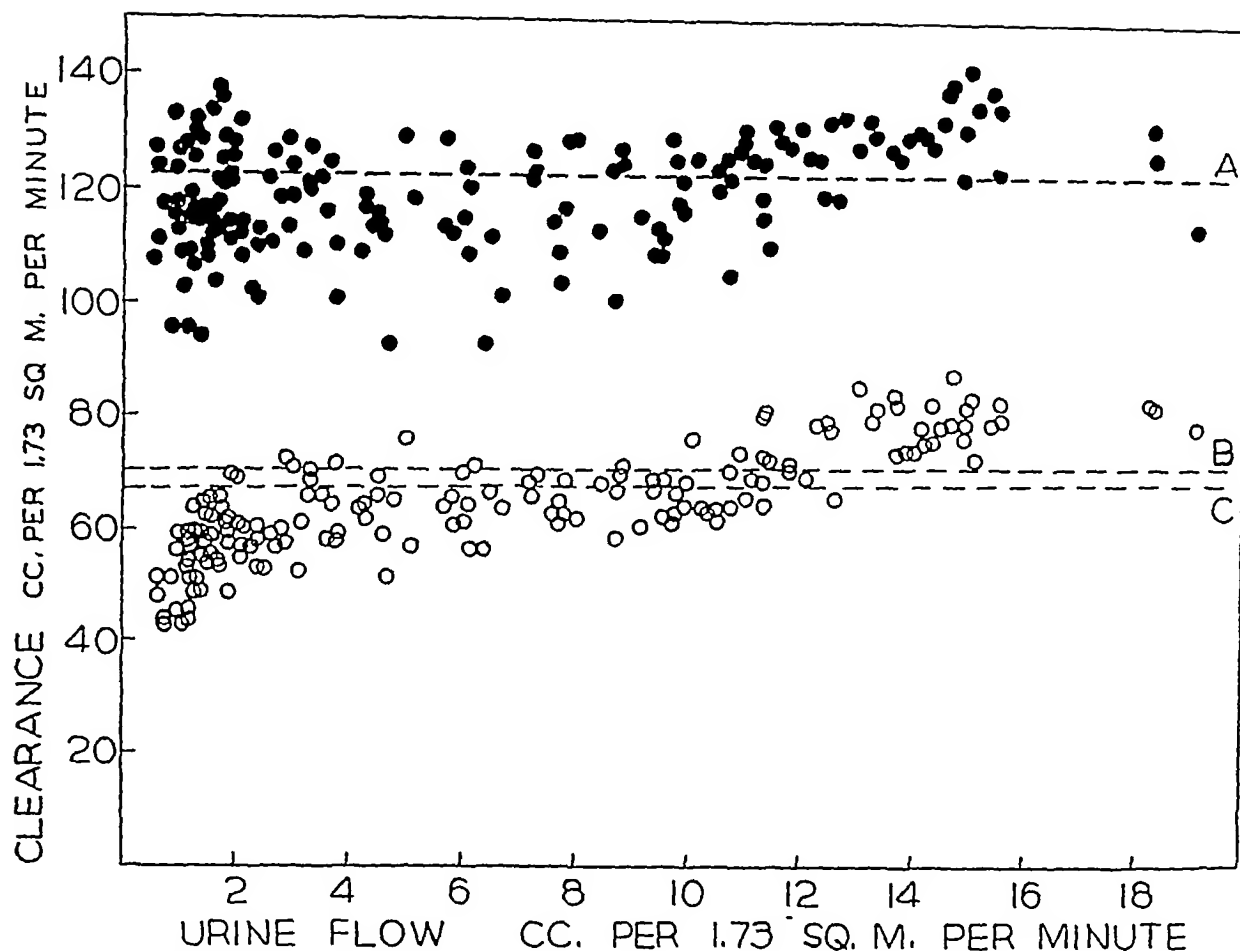
FIG 2   186 SIMULTANEOUS INULIN (*dots*) AND UREA (*circles*) CLEARANCES IN 9 NORMAL SUBJECTS DURING
FALLING OR CONSTANT URINE FLOWS

Each datum is a single clearance period   Line *A*   mean plasma inulin clearance (122 5 cc) in normal man
(34), line *B*   mean plasma urea clearance (70 5 cc.) reported by Smith, Goldring and Chasis (34), line *C*
mean plasma urea clearance (67 5 cc.) calculated from Möller, McIntosh and Van Slyke's (20) value for whole
blood, assuming 60 per cent plasma

difference between the urea and inulin clearances
in normal man is indicated by the small amount
of NH_3 excreted normally, we have demonstrated
this specifically by 14 observations on Subject
T G, which show that the urea + ammonia —
N clearance is not significantly greater than the
urea clearance, either at high or low urine flows

Reabsorption of urea

In discussing the reabsorption of urea it is
necessary, for reasons which will be apparent, to
divide the data into two categories, first, clear-
ances determined when the urine flow is decreas-
ing or has reached a constant value, and second,
clearances determined when the urine flow is in-
creasing in consequence of water diuresis

Inspection of the absolute urea clearance, as
portrayed in Figures 1 and 2, shows that there is
a progressive increase in the urea clearance as the
urine flow increases from low to high values
This is owing in part to the increase in glomerular
filtration and in part to diminished reabsorption
Variations in the latter can best be examined by
considering the urea/inulin clearance ratio, since
1 — urea/inulin clearance ratio gives directly the
fraction of filtered urea which has been re-
absorbed   For the examination of the effect of
urine flow upon this reabsorbed fraction it is con-
venient to relate the latter to the U/P ratio of
inulin, since this ratio expresses the degree to
which the glomerular filtrate has been concen-
trated by the reabsorption of water   The data on

The effects of rising urine flow on the urea/inulin clearance ratio were examined in three subjects Observations on Subjects E B and T G are shown by the solid triangles in Figure 3 (For similar observations on Subject C B see Figure 23 (33)) Abrupt acceleration of urine flow leads to an increase in the urea/inulin clearance ratio, this increase being out of proportion to the anticipated effect of changing inulin U/P ratio, as observed when the urine flow is decreasing or constant This transitory elevation of the urea clearance, relative to the inulin clearance, is probably identical in origin with the similar phenomenon in the dog (27) It can be elicited both at low and intermediate urine flows, and by the intravenous administration of hypertonic sodium sulphate, and it may occur after the administration of water even though the decrease in inulin U/P ratio is negligible It does not appear to be a result of error in calculating clearances introduced by dead space in the tubules, ureters, or bladder, since the plasma concentration of both inulin and urea was practically constant during these observations, the effect of flushing out dead space on the U/P ratio of both substances should therefore be identical

We feel justified, in considering the problem of urea reabsorption, in assuming that the relationship between the inulin and urea clearance as observed during falling or constant urine flow represents the primary physiological relationship, and we set aside the elevation of the urea clearance during acceleration of the urine flow as a subordinate phenomena which cannot profitably be discussed at this time.

In the upper part of Figure 4 there is presented a mass plot of data on the 10 normal subjects examined by us The line B represents the best straight line that can be drawn through these data, excluding the points at the extreme right In considering the significance of this line it must be recognized that the lowest inulin U/P ratios observed by us in normal man at or after the period of water diuresis, range from 10 to 6 (i e, urine flows of 12 to 20 cc. per minute) The highest inulin U/P ratio obtainable with our technique of examination (i e, with intravenous saline at 4 0 cc. per minute) is about 200 (urine flow = 0 6 cc. per minute), though much *higher* ¬< can ¬

FIG. 3 THE UREA/INULIN CLEARANCE RATIO IN SUBJECTS E. B AND T G IN RELATION TO THE DEGREE OF CONCENTRATION OF THE GLOMERULAR FILTRATE AS INDICATED BY THE INULIN U/P RATIO

Each point is a single clearance period. The circles are observations on falling or constant urine flows and the triangles are observations immediately after the administration of 1500 cc. of water

Subjects E B and T G are presented in this manner in Figure 3

At inulin U/P ratios of 6 to 10, which are the lowest obtainable in normal man during water diuresis (i e, at urine flows of 12 to 20 cc. per minute), the urea/inulin clearance ratio does not on the average rise above 0 60, i e at maximal diuresis 40 per cent of the filtered urea is reabsorbed As the U/P ratio of inulin increases (i e, as the urine flow decreases), the urea/inulin clearance ratio decreases showing that a larger fraction of filtered urea is reabsorbed at low than at high urine flows Although a fairly regular relationship exists between the log of the inulin U/P ratio and the fraction of urea reabsorbed the large scatter of the data suggests that the reabsorptive process is influenced by factors other than the ultimate degree of concentration of the glomerular filtrate. Two such subsidiary factors will be indicated later

NORMAL

GLOMERULONEPHRITIS
INULIN CLEARANCE
PER CENT OF NORMAL
● 80-130
◐ 60- 80
▢ 40- 60
◼ 20- 40
▲ 4- 20

Legend for Figure 4 on opposite page

obtained without infusion[5] Except for slight differences in slope, line B corresponds very closely to the recent data of Shannon (29) on Dogs C and G (which appear to be typical), the urea/inulin clearance ratio in these two dogs and in our data having a common value of 0 56 at an inulin U/P ratio of 20 So it may be said that between inulin U/P ratios of 6 and 200 (urine flows of 20 and 0 6 cc.) man and dog behave so nearly alike in respect to the reabsorption of urea that the underlying causes may be considered to be essentially the same in both species

Shannon (27, 29) has presented arguments against the belief that the reabsorption of urea in the dog is owing to a single process of passive diffusion determined by the degree of concentration of the glomerular filtrate and the time of contact of the tubular urine with the renal tubules, as calculated from the final composition of the urine He has recently shown that when diuresis is induced by the continuous intravenous infusion of glucose, Na_2SO_4, or urea, the urea/inulin clearance ratio rises towards 1 0 following the path indicated by line A in Figure 4 At inulin U/P ratios of 1 5 to 2 0 (urine flows of 50 cc. per minute in a 20 kgm dog) he has obtained urea/inulin clearance ratios as high as 0.945

We have not attempted to make observations during forced diuresis in man, but since the excretion of urea in dog and man are so similar in all other respects there is every reason to believe that the human kidney would respond in the same manner as the dog kidney We therefore present the two lines, A and B in Figure 4, as

[5] Because of the insolubility of inulin it is advisable to keep the urine flow above 1.0 cc. per minute, or to reduce considerably the plasma concentration of inulin

representing the approximate relationship in the normal human kidney of urea reabsorption to the inulin U/P ratio at values of the latter ranging from 1 to 200 (i e , at urine flows between 122 and 0 6 cc. per minute)

Physiological basis of urea reabsorption

There is no evidence that urea is actively reabsorbed in any mammal, such active reabsorption is known to occur in the elasmobranch fishes, where the concentration of urea in the blood is very high (2000 to 3000 mgm per cent), the concentration in the urine is invariably less than in the blood, and the concentration in the urine may rise and approach the concentration in the blood during diuresis But in the elasmobranch fishes a special segment of the tubule, not present in other vertebrates, is apparently responsible for this active reabsorption (31) All the evidence in the mammals points to the conclusion that urea is *reabsorbed passively*, however *multiform or complex* this reabsorption, and, indeed, it appears that this reabsorption can be explained entirely by diffusion in consequence of a concentration gradient established by the reabsorption of water, providing it is assumed that the reabsorption of water takes place in at least two stages and at different loci in the tubule.

Although present methods do not afford a means of distinguishing separate processes of water reabsorption, Smith (33) has suggested that at least two such processes exist One consists of the isosmotic reabsorption of water in the proximal tubule, which is accompanied by, or made possible by, the simultaneous reabsorption of chloride, glucose, and other substances Since this process accounts for about 100 cc out of the 120

FIG. 4   (ABOVE) 225 OBSERVATIONS ON 10 NORMAL SUBJECTS AT URINE FLOWS FROM 20 DOWN TO 0.5 CC. PER MINUTE (THE NORMAL RANGE OF DIURESIS)

Line B the best straight line through these data Line A the relationship to be expected at urine flows of 20 to 122 cc. per minute, based on observations on the dog (29)

It is suggested that line A represents one reabsorptive process related to the obligatory reabsorption of water in the proximal segment, and line B a second reabsorptive process related to the facultative reabsorption of water in the thin limb and distal segment. The former process cannot be abolished in the normal kidney by water diuresis.

(BELOW) 167 OBSERVATIONS ON 22 SUBJECTS WITH GLOMERULONEPHRITIS

Lines A and B as described above. At any given inulin U/P ratio the fraction of urea reabsorbed is essentially the same in the nephritic kidney at all stages of the disease as in the normal. Partial impairment of obligatory reabsorption of water at a time when facultative reabsorption is still present tends to obl ᵤ between A and B

cc of glomerular filtrate which is never excreted by the normal kidney, Smith called it the "obligatory" reabsorption of water   The second process of water reabsorption appears to occur in the distal portions of the tubule, it is in this process that the final urine is concentrated and raised to osmotic pressures greater than the blood, and since it is by variations in this process that the rate of water excretion (urine flow) is controlled, the process was designated as "facultative" reabsorption

We are inclined to attribute that moiety of urea reabsorption (30 to 40 per cent) which occurs at inulin U/P ratios of 10 to the obligatory reabsorption of water, and the further reabsorption of urea at inulin U/P ratios above 10 to the facultative reabsorption of water   In doing so, we are joining with Shannon (29) in a similar identification in the dog   Since the obligatory reabsorption of water cannot be reduced by water diuresis in normal man (i e, the inulin U/P ratio cannot be lowered much below 10), the urea clearance cannot be raised by water diuresis higher than about 70 per cent of the filtration rate

It must be recognized that a reduction in the filtration rate, whether resulting from lowered arterial pressure, oligemia, or other causes, can lead to an increased reabsorption of urea, possibly because it prolongs the time for diffusion (27) Variations in the filtration rate relative to the mass of normal tubular tissue, together with variations in the obligatory reabsorption of water, may in part account not only for the scatter of the data in Figure 4, but also for the elevation of the urea clearance on rising urine flows, as illustrated in Figure 3, and the apparently excessive depression of the urea/inulin clearance ratio at high inulin U/P ratios in both normal and nephritic subjects   Although of considerable importance, it is impossible at the present time, and unnecessary for our present purposes, to treat these aspects of the problem quantitatively

Inulin and urea clearances in subjects with glomerulonephritis

We present 167 simultaneous urea and inulin clearances in 22 subjects, 7 of whom were examined during a first attack of diffuse glomerulonephritis and 15 in the chronic stage   Some

of the latter were examined during an acute exacerbation   There appears to be no necessity for considering these subjects in detail, essential data are given in Table I, and the urea/inulin clearance ratio in relation to the inulin U/P ratio is presented in the lower part of Figure 4

It is to be noted that our observations on subjects with glomerulonephritis have all been made under conditions of water plethora, and on the descending limb or close to the peak of maximal water diuresis   Our data are therefore of such a nature as to reveal a change in the minimal inulin U/P ratio

It is immaterial to this discussion whether or not the urine during maximal diuresis is dilute, in the sense of having a low specific gravity, since the specific gravity depends upon the variable reabsorption of chloride, glucose, and other substances   The important point is the extent to which the glomerular filtrate is concentrated by the obligatory reabsorption of water

As said above, in the normal subject the minimal inulin U/P ratio has never been observed to fall below 6, and it frequently cannot be reduced below 10   This is in sharp contrast to the nephritic kidney in which the reabsorptive function of the tubule has been impaired by disease Reference to the mass plot in the lower part of Figure 4 shows that, with advancing destruction of renal tissue, the minimal inulin U/P ratio falls, and ultimately reaches such low values as 2 0 It must be concluded from this fact that during the course of disease the obligatory reabsorption of water is for some reason impaired   In advanced nephritis there is frequently a continuous excretion of base and chloride,[6] and it is possible that it is the failure of electrolyte reabsorption and related processes that leads, by osmotic obstruction, to reduction in the obligatory reabsorption of water   But whatever the cause, as the minimal inulin U/P ratio falls from 10 to 2, the reabsorption of urea decreases and the urea/inu-

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[6] This circumstance perhaps contributes to the fixed specific gravity of the urine, characterized clinically as isosthenuria. Our observations were not designed to examine the effect of disease upon the facultative reabsorption of water (i e, the production of a hypertonic urine), it is presumably the failure of this process that leads to the clinical conditions of polyuria and hyposthenuria.

TABLE I

Inulin and urea clearances in subjects with acute or chronic diffuse glomerulonephritis

Subject	Days after first observation	Plasma urea	Clearance * per 1 73 sq m.	
			Inulin	Urea
		mgm per cent	cc, per minute	cc, per minute

ACUTE DIFFUSE GLOMERULONEPHRITIS

Subject	Days after first observation	Plasma urea	Inulin	Urea
S. M		62.3	57 6	31.3
O M †		40 4	71 9	43 0
T B		69 0	46.2	26 6
T B	7	50 4	61 3	45 4
T B	14	55 1	61 6	44.2
T B	25	44 7	71 1	47.5
T B	36	41 1	81 9	55 7
R. M		30 2	97 9	57.2
J G		27 5	108 2	64 4
P S		33 6	88.3	35 8*
I E		17 1	130 6	59 1
J E	5	18 5	118.3	57 9
J E	12	20 4	122 1	62.2
J E	35	22 5	122 3	58 9
D G		34 6	89 1	39.5*
D G	5	33 3	88.5	39 6
D G	21	23 7	117 8	68 7

CHRONIC DIFFUSE GLOMERULONEPHRITIS

Subject	Days after first observation	Plasma urea	Inulin	Urea
M J		37 2	28 8	23.2
K. S		75 7	17.3	14 7
T S.		254 6	4 4	3 4
T S	7	266 0	4.5	3 8
T S	21	292 0	4 7	3 7
T S	28	301 0	4 9	4 1
T S	49	387 5	4 8	4 0
T S	65	421 0	3 5	2 8
A. C.†		98 7	9 8	7 9
S N		34 5	49 6	34 2
E M		125 0	13 6	10 0
D G	336	23 6	136.3	64.5
C. P		153 9	18 4	14 7
D W		298 0	5 5	4 7*
J D		142.2	13 8	11 4
L. S		18 1	105 6	45 4
L. S	12	21 1	143 5	60 6
H S.		87 6	15 8	12 5
H S	365	228 2	6 2	5 4
L. S		145 4	13 9	10 7
T L.		70 0	60 8	31 4
T L	6	66 0	66 7	32 9*
T L	240	72 4	22.2	15 1*
T L	256	70 0	21 8	15.5*
T L	334	81 4	17 5	13 6*
R. D		40.3	72 4	57.5

† These subjects were in congestive heart failure at the time of examination Their urea/inulin clearance ratios in relation to the inulin U/P ratios were in agreement with all the other subjects studied

Each clearance figure is the average of four consecutive urine collection periods with average urine volume of 1 0 to 1.5 cc. per minute where marked * otherwise above 1 5 cc. per minute.

lin clearance ratio rises towards 1 0 along the general course indicated by line A. In short, at any given inulin U/P ratio the reabsorption of urea proceeds in the nephritic kidney just as it would in the normal kidney

Certain apparent exceptions to this statement are, we believe, subject to explanation. The data on the nephritic kidney are not distributed in two distinct relationships (*A* and *B*) in Figure 4, but fall with fair uniformity along a single rectilinear relationship This transposition is to be expected if impairment of the obligatory reabsorption of water (which would lead to movement upward along line *A*) occurs at a time when the facultative reabsorption (which leads to movement to the right along line *B*) is still capable of concentrating the urine Hence an early reduction in the obligatory reabsorption of water would tend to obliterate the angle between line *A* and *B* The tendency of the data to fall below line *B* at high inulin U/P ratios may be associated with a reduction in filtration rate, either due to prerenal deviation of water or glomerular dysfunction, at a time when the mass of functional tubules is still *essentially normal*

The above interpretation rests upon the assumption that the inulin clearance is a trustworthy index of the rate of glomerular filtration in the nephritic kidney It might be argued that, even though there is no reabsorption of inulin in the normal kidney, such reabsorption occurs in the nephritic kidney, and that the convergence of the urea and inulin clearances in the latter is a result of the simultaneous diffusion of these substances out of the tubular urine. Against this belief there may be advanced the facts that the diffusion coefficient of urea is seven times as great as that of inulin (4), and the difference in penetrating power must be even greater where protoplasmic barriers are involved The normal tubule is apparently impermeable to inulin, and its permeability to urea (one of the most diffusible substances so far as living tissues are concerned) is of a very low order If an increase in permeability occurred, urea should escape first, and seven times as rapidly as inulin, and the fraction of urea reabsorbed should show a detectable increase Moreover, the urea/inulin clearance ratio should decrease more rapidly with increasing inulin U/P ratio, in the nephritic kidney than it does in
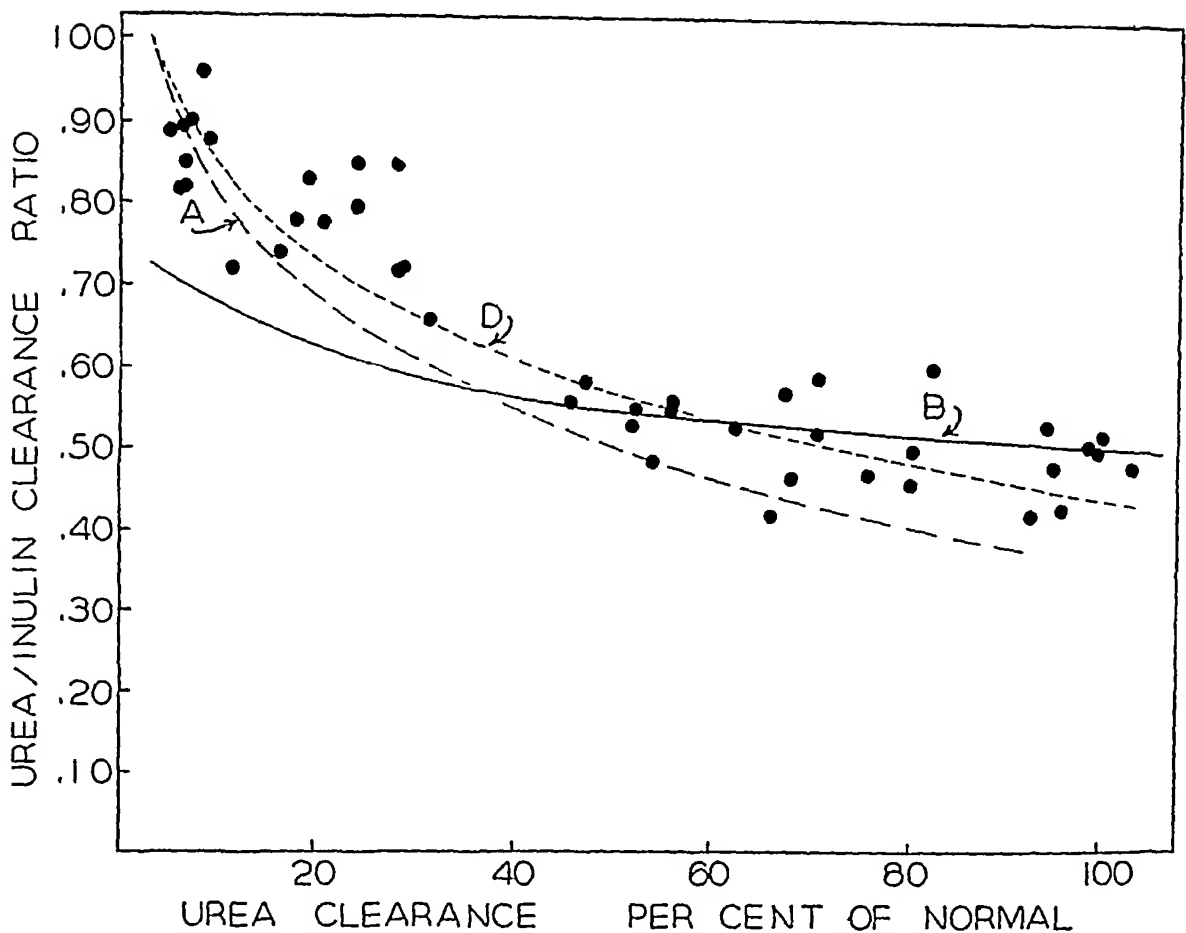
Fig 5   The Urea Clearance When $V = 20$ cc. Per Minute (43 Observations) in 22 Subjects with Glomerulonephritis

Since the value of the urea clearance depends on the urine volume, the use of unselected urea clearances for the above comparison introduces unnecessary deviation, and for the construction of this figure we have first plotted all our urea clearances against the urine flow Treating each group of subjects having approximately the same inulin clearance separately, we have drawn smooth curves through the resulting scatter diagram and from these curves interpolated the value of the urea clearance for each subject when the urine flow is 20 cc per minute. In the early stages of disease the urea clearance ($V = 20$ cc. per minute) has an average value of 50 per cent of the inulin clearance, as renal impairment progresses it rises to 90 per cent of the inulin clearance. Lines A and B show the relationships calculated from the corresponding lines in Figure 4 Line D (not shown in Figure 4) is the relationship calculated from a single median line through all the data on nephritics, as shown in that figure.

normal All the data are contrary to these expectations In our series we have not observed a nephritic subject, such as has been reported by others (14, 2, 5, 39), in whom the urea clearance falls to low values relative to the glomerular clearance It may be that in such subjects the glomerular clearance is abnormally high, that the filtration rate relative to the residual functional tubular tissue is abnormally low, or that unusual reabsorption of urea occurs by the mechanism responsible for the transient, complete reabsorption which has been reported in the dog (11, 37)

But insofar as the subjects examined by us are typical of the course of glomerulonephritis, it is clear that the reabsorption of urea decreases progressively as glomerular function is reduced The practical significance of this fact is effectively illustrated in Figure 5, where the urea/inulin clearance ratio in the nephritic subjects examined here is plotted against the urea clearance expressed as per cent of the normal value [r]

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[r] The tendency of the creatinine and urea clearances to converge in advanced nephritis is evident in the reports

The above facts are clearly contrary to the view that the elevation of the blood urea in glomerulonephritis is owing to increased reabsorption of urea. Our results indicate that this elevation is owing to decreased filtration, with actually decreased reabsorption. In principle, it appears that the blood urea, all other things being equal, should vary inversely as the urea clearance, such an inverse relationship is adequately demonstrated by the well known scatter diagrams relating the blood urea to the urea clearance which have been presented by MacKay and MacKay (18) and Van Slyke, McIntosh, Möller, Hannon and Johnston (35)

"Standard" and "maximum" urea clearances

Inspection of Figures 1 and 2 shows that our data conform roughly to the "standard" and "maximum" clearance concept of Möller, McIntosh and Van Slyke (20) In the light of the further analysis submitted here, however, it is clear that the standard clearance is an approximation, while the "maximum" clearance neglects the effect of urine flow on the reabsorption of urea at inulin U/P ratios below 60 But since the effect of urine flow is slight when the latter is 2 0 cc or above, it appears advisable in the interests of simplicity to adhere to the uncorrected clearance in utilizing the excretion of urea as an index of renal function It is not possible, from our present data, to comment on the accuracy of the use of $\sqrt{V}$ in the calculation of the "standard" clearance but it would seem advisable for physiological reasons to maintain the urine flow above 1.5 cc. wherever possible, since at flows below that level complicating factors (dehydration, lowered filtration rate, etc) may vitiate any empirical mathematical correction

of several observers, and has been commented upon by Winkler and Parra (39) This convergence is explicable in terms of decreased reabsorption of urea due to reduction in the degree of concentration of the glomerular filtrate. There is no evidence that the permeability of the glomerular membranes is reduced in nephritis in such a manner as to prevent the free filtration of inulin but not urea. The continued excretion of albumin, the convergence of the creatinine and urea clearances, and the fact that the urea clearance never exceeds the mulin clearance may be advanced as arguments against this supposition.

SUMMARY

The urea clearance has been examined in 10 normal subjects and 22 subjects with glomerulonephritis, with special reference to the degree of concentration of the glomerular filtrate as indicated by the simultaneous inulin U/P ratio

Urea is invariably reabsorbed to some extent from the glomerular filtrate, whether this is at the normal level or is reduced by disease This reabsorption is interpreted in terms of an hypothesis, based upon independent evidence, in which it is posited that water reabsorption occurs in two stages, one in the proximal tubule and one in the thin limb and distal tubule

At any inulin U/P ratio (i e, degree of concentration of the glomerular filtrate) the reabsorption of urea proceeds in the nephritic kidney essentially as it would in the normal kidney As the capacity to reabsorb water is impaired by disease, the fraction of urea reabsorbed decreases, so that the urea clearance approaches the rate of glomerular filtration

In none of the subjects examined was there evidence of increased back-diffusion of urea, the elevation of the blood urea in nephritis being a result solely of the reciprocal relationship between this term and the urea clearance, as expected in principle.

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THE EFFECT OF TOTAL SYMPATHECTOMY ON THE OCCURRENCE OF SHOCK FROM HEMORRHAGE

By N. E. FREEMAN, S. A. SHAFFER, A. E. SCHECTER AND H. E. HOLLING[1]

WITH THE TECHNICAL ASSISTANCE OF N. E. MAREAN

(*From the Harrison Department of Surgical Research School of Medicine University of Pennsylvania Philadelphia*)

Shock has been differentiated from hemorrhage on the basis of hemoconcentration, negative reaction to blood transfusions, and pathological changes in the tissues (1) In hemorrhage dilution of the blood occurs while concentration takes place in shock After simple loss of blood, prompt recovery follows transfusion, whereas in severe shock, the administration of blood is frequently unavailing It has been held that the tissues are anemic after hemorrhage while the pathological picture of shock is that of " dilatation and engorgement of capillaries and vessels ' (2) Blalock, however, in a convincing series of experiments (3), showed that the classical picture of shock with hemoconcentration, negative reaction to transfusion, and pathological changes in the tissues, could be produced by hemorrhage alone. In his experiments on dogs under local anesthesia, the blood pressure was maintained at a low level for several hours by means of hemorrhage At the end of this time, in spite of the fact that all the blood which had been removed was reinjected, the blood pressure continued to fall and the animal died The present experiments were performed to investigate further the mechanism through which shock was produced by hemorrhage.

It is recognized that vasoconstriction takes place after hemorrhage and this reaction has been described as protective (4) By means of con traction of the vessels in the presence of hemor rhage, the blood pressure is maintained at a level compatible with life After removal of the vaso constrictors by complete sympathectomy, cats under anesthesia are unable to tolerate as great a loss of blood as normal animals (5) In a former communication (6), it was shown, on the other hand that intense and prolonged vasoconstriction caused a reduction in blood volume and

shock in the experimental animal This apparent paradox was attributed to a reduction in the volume flow of blood to the peripheral tissues produced by constriction of the arterioles The hypothesis was advanced that vasoconstriction enabled the organism to adjust to the immediate crisis If this reaction were so intense and protracted, however, as to reduce the nutrient flow to the tissues, shock would be produced.

The reaction of normal dogs to hemorrhage was, therefore, compared with that of completely sympathectomized dogs Particular attention was paid to the function of vasoconstriction in main taining blood pressure, and at the same time in restricting the flow of blood to the peripheral tissues Arterial blood pressure was correlated with the volume flow of blood through the paw

Death or recovery of the dog was used as the ultimate criterion of shock. Concentration of the peripheral blood, alterations in the oxygen and carbon dioxide contents of the arterial and venous blood, and the reactions of the dogs to transfusions were noted At the end of the experiments various tissues were examined histologically

METHODS

Dogs were used which weighed between 11 and 22 kilograms. Under aseptic precautions cannulae were inserted into the carotid artery and the jugular vein or the femoral artery and vein. Pain was prevented by the use of novocain in the operative wounds The blood pressure was determined by means of a Hürtle manom eter calibrated with a mercury manometer Blood was obtained for analysis of the oxygen content and capacity and carbon dioxide content from the carotid artery and from the right heart. The blood was taken under oil chilled, and analyzed at the conclusion of the experi ment. The oxygen content and capacity and the carbon dioxide content were determined by the method of Van Slyke and Neill (7) In certain of the experiments the plasma volume was determined by the method of Greger

TABLE I

Data compiled from all the experiments on hemorrhage in the normal and sympathectomized dogs

Dog number	Weight	Plasma volume	Initial bleeding	Per cent of body weight	Time of initial bleeding	Total hemorrhage	Amount transfused	Hemoglobin			Average after hemorrhage*			After transfusion		Result
								Start	Maximum dilution	Concentration after dilution	Blood pressure	Blood flow	Duration	Blood pressure	Blood flow	
	kilos	cc.	cc		hours	cc.	cc	per cent	per cent	per cent	mm Hg	cc. per 100 cc paw volume per minute	hours	mm Hg	cc. per 100 cc paw volume per minute	
NORMAL DOGS—DIED																
25	14.2	1010	705	5.4	3.6	705	820	106	91	117	67	0.9	6.6	60	0	Died
16	18.8		814	4.3	1.4	859	845	78	64	83	70	1.5	2.1	50	1.0	Died
751	13.6	742	1000	7.3	1.9	1153	820	115	63	95	60	0.4	4.6	85	0.8	Died during reinjection
767	17.4	800	907	1.2	1.9	1032	173	110	60		62	0.9	2.4	60		Acute cardiac failure
570	14.7	718	543	3.8	2.8	703	650	100	83	92	49	0.1	3.6	40	0	Died
2	14.7		1059	7.5	1.4	1055	460				42	0.1	2.0	30		Died during reinjection
540	22.0		1000	4.5	1.4	1000	1030				35	1.6	3.5	42	2.5	Died
NORMAL DOGS—RECOVERED																
540	22.0	800	940	4.5	1.8	1317	990	84	62		60	2.9	2.2	130	20.4	Recovered
611	11.6		650	5.9	1.6	850	845	103	61		63	2.1	6.0	95	12.2	Recovered—sacrificed
SYMPATHECTOMIZED DOGS																
395	14.7	670	512	3.5	1.5	641	436	75	56	65	52	1.2	3.9	120	9.6	Recovered—sacrificed
418	14.0	900	458	3.5	1.0	691	660	82	65		55	1.9	5.6	120	16.2	Recovered—living
14	14.0		604	3.6	1.0	591	513				50	0.8	4.7	163	33.0	Recovered—living

* The average blood pressure and blood flow, after hemorrhage was calculated with a planimeter

sen (8) ² Hemoglobin estimations were made by the Sahli method on blood taken from the ear The volume flow of blood through the hind paw was determined by the plethysmographic method previously described (9) The temperature of the water bath in which the paw was immersed was kept between 36° and 39° C

The dogs were bled from the carotid or femoral arteries into a sterile container which contained 2 5 per cent sodium citrate solution The final dilution of citrate in blood was 0 25 per cent

After control observations on blood pressure and blood flow were made, the dogs were bled at frequent intervals until the blood pressure and blood flow were reduced to low levels from which rapid recovery did not occur This period of hemorrhage is considered in Table I to be the time of initial bleeding It varied from 1 4 to 3 6 hours in the normal dogs and 1 0 to 1 5 hours in the sympathectomized dogs After this period of initial bleeding, the dogs were allowed to continue with a low blood pressure and diminished blood flow as long as possible If the blood pressure or blood flow started to rise, blood was again withdrawn When the blood pressure started to decline spontaneously or if the dog showed signs of loss of consciousness, small amounts of blood were reinjected

² The blue dye T-1824 was obtained through the kindness of Doctor Gregersen

Blood, which had been removed under aseptic precautions from donor dogs, was also injected in two of the experiments so that the total amount of blood which these animals received after hemorrhage was larger than the amount which was removed during the experiment.

Complete sympathectomy was performed by removal of the paravertebral sympathetic ganglia in three stages as described by Cannon (10) The sympathectomized dogs had fully recovered from the effects of the operative procedures at the time they were used in the experiments

RESULTS

In the normal dog, after the blood pressure and blood flow had been reduced for a period of hours by hemorrhage, shock was produced This condition was characterized by concentration of the peripheral blood, negative reaction to blood transfusion, and failure to recover Figure 1 illustrates this reaction In spite of the fact that the blood pressure at no time fell below 60 mm Hg the blood flow was reduced to a low level At the end of three and one-half hours, the blood pressure failed to rise and the blood flow started to fall spontaneously Blood was then
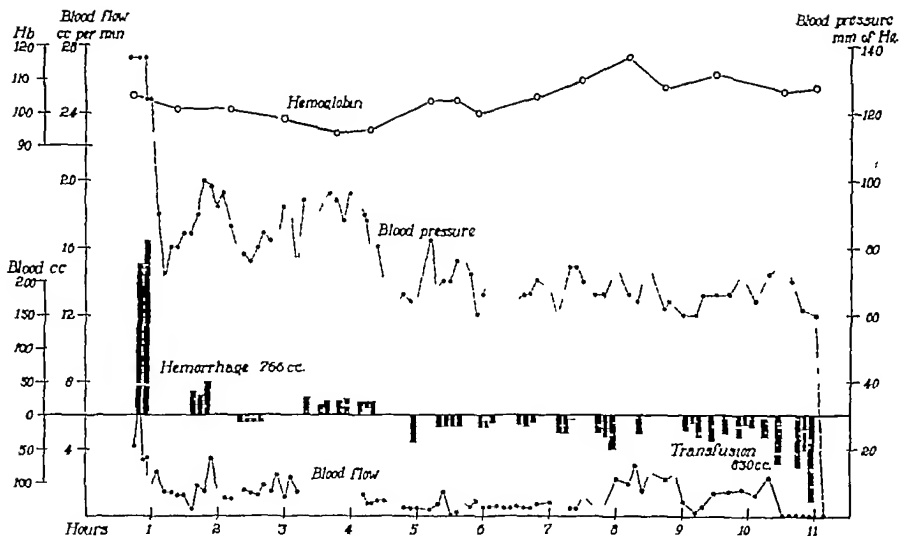
FIG 1 EFFECT OF HEMORRHAGE, FOLLOWED BY TRANSFUSION ON THE VOLUME FLOW OF BLOOD THROUGH THE HIND PAW THE BLOOD PRESSURE, AND THE HEMOGLOBIN CONTENT OF THE VENOUS BLOOD IN A NORMAL DOG DOG 25 14.2 KILOGRAMS.
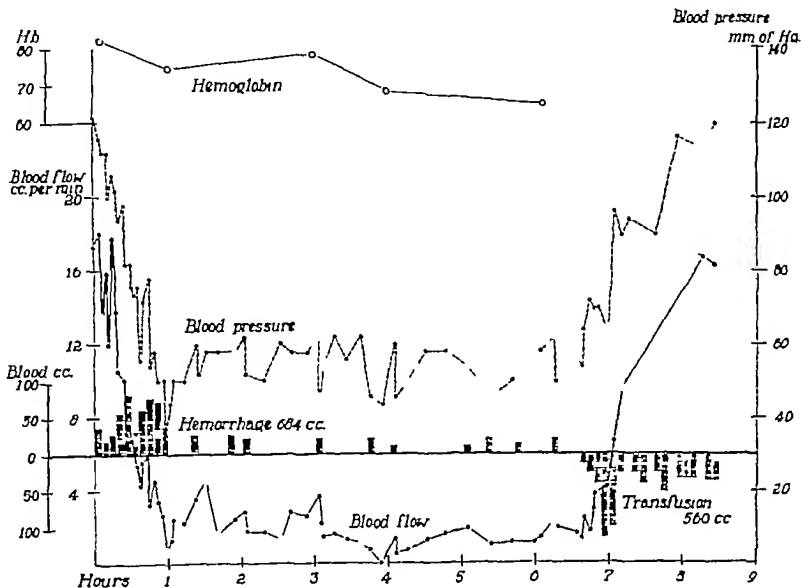
FIG. 2 EFFECT OF HEMORRHAGE, FOLLOWED BY TRANSFUSION ON VOLUME FLOW OF BLOOD THROUGH THE HIND PAW THE BLOOD PRESSURE, AND THE HEMOGLOBIN CONTENT OF THE VENOUS BLOOD IN A SYMPATHECTOMIZED DOG. DOG 418 14 0 KILOGRAMS

Liver
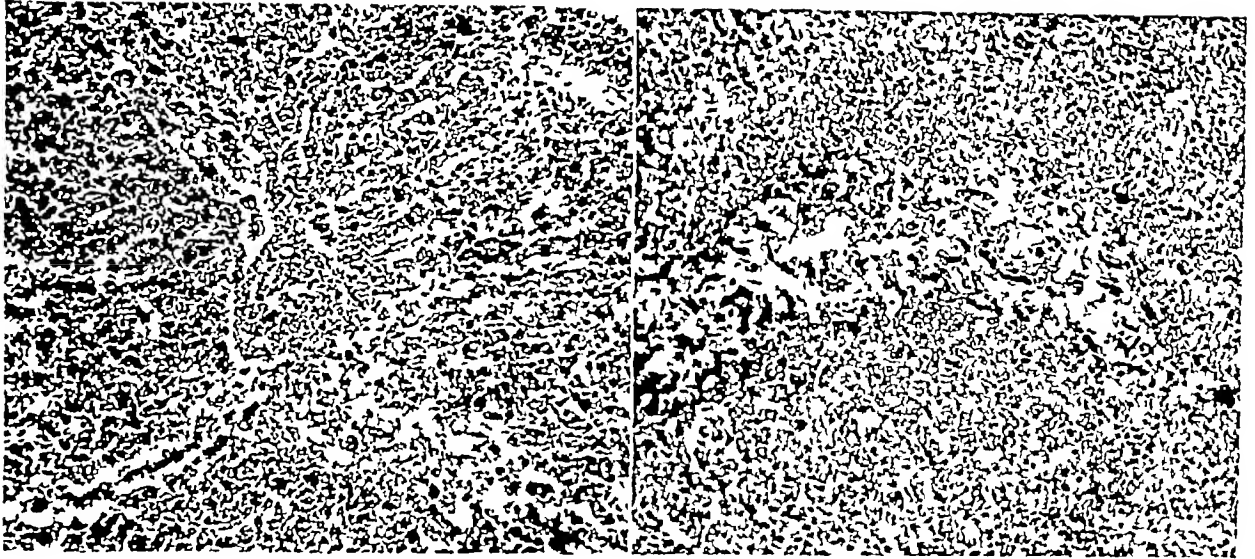
A

B

Duodenum

C

D

Fig 3 Histological Findings in the Liver (*A*) and Duodenum (*C*) of One of the Normal Dogs Which Died in Shock in Contrast to the Liver (*B*) and Duodenum (*D*) of One of the Sympathectomized Dogs Which was Sacrificed after Recovery

slowly reinjected   Over a period of six hours, although 64 cc more blood were injected than had been removed, the blood pressure and blood flow failed to return to the original level   At the conclusion of the injection the dog died

In the sympathectomized dog, as shown in Figure 2, although the blood pressure was reduced to *below* 60 mm Hg and maintained at this level for six hours before the reinjection of blood, the blood flow was not reduced to as great an extent, and recovery took place   Instead of hemoconcentration, dilution of the blood took place, even though the blood pressure was at a lower level than in the normal dog   The prompt response

of blood pressure and blood flow to the reinjection of blood stands in contrast to the absence of response in the normal dog At the conclusion of the experiment, the sympathectomized dog recovered completely and ran back to its cage

The results obtained in nine experiments on normal dogs and three experiments on sympathectomized dogs are summarized in Table I The amount of blood loss in proportion to the

blood was minor but there were extensive patches of necrosis of the liver cells In the duodenum of the shocked dog (C) the superficial portion of the mucous membrane had disappeared This observation accorded with the fact that during the periods of low blood pressure, the shocked dogs always had profuse bloody diarrhea in which sloughs of the mucous membrane could be made out. The mucous membrane of the duodenum in
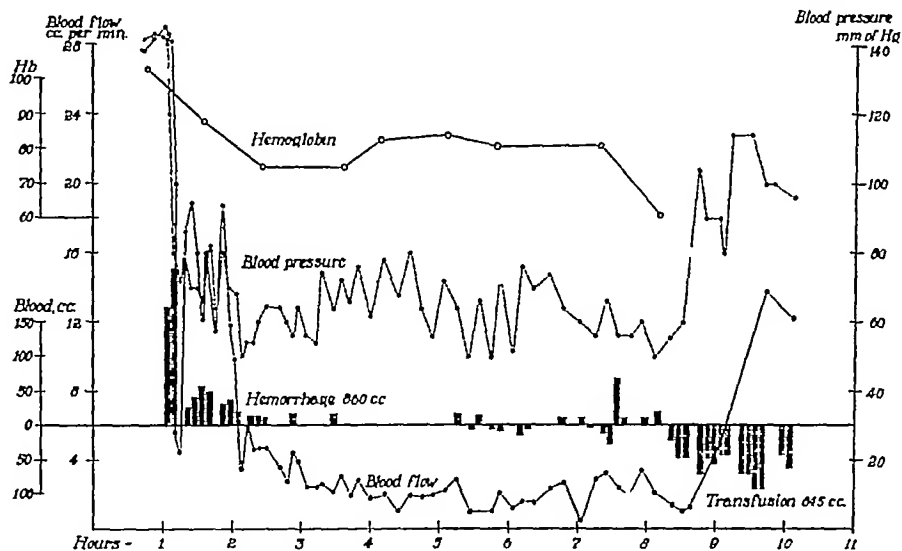
FIG 4 EFFECT OF HEMORRHAGE, FOLLOWED BY TRANSFUSION ON THE VOLUME FLOW OF BLOOD THROUGH THE HIND PAW THE BLOOD PRESSURE, AND THE HEMOGLOBIN CONTENT OF THE VENOUS BLOOD IN A NORMAL DOG WHICH RECOVERED. DOG 641, 11 6 KILOGRAMS.

body weight was greater in the normal dogs (5 4 per cent) than in the sympathectomized dogs (3 6 per cent) The time necessary to reduce the blood pressure to a low level in the normal dogs (2 1 hours) was also greater than that required in the sympathectomized dogs (1.2 hours)

In Figure 3 are shown the contrasting histological pictures of one of the normal dogs which died in shock and one of the sympathectomized dogs which was sacrificed after recovery The liver of the dog which died in shock (A) showed tremendous engorgement with blood and some evidence of degeneration of the liver cells In the sympathectomized dog (B) engorgement with

the sympathectomized dog (D) was preserved intact although there was tremendous engorgement of blood in the vessels

In two of the normal dogs, recovery took place, even though the blood pressure had been reduced to a low level for six hours The amount of hemorrhage was equivalent to that in the dogs which went into shock. Figure 4 illustrates the reaction in one of the dogs It can be seen that the blood flow through the paw was not reduced to as great an extent as in the shocked dogs Although concentration of the blood took place at one time in the experiment considerable dilution occurred later
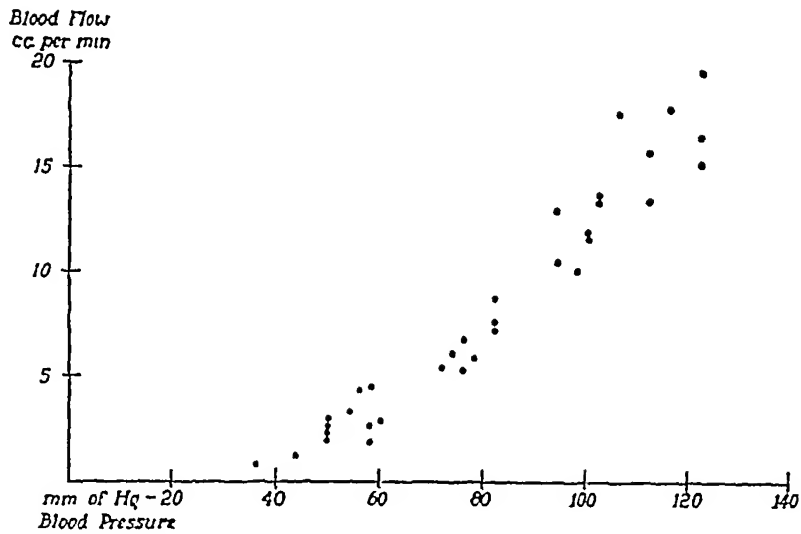
FIG 5 EFFECT OF REDUCING BLOOD PRESSURE BY MEANS OF HEMOR-
RHAGE ON VOLUME FLOW OF BLOOD THROUGH THE HIND PAW OF A SYMPA-
THECTOMIZED DOG
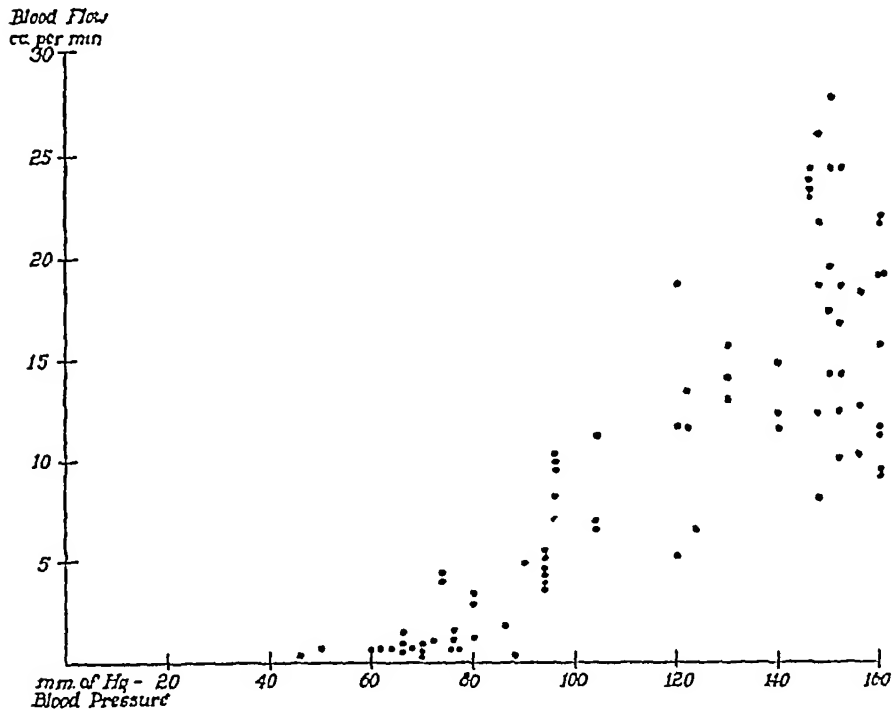
FIG 6 EFFECT OF REDUCING BLOOD PRESSURE BY MEANS OF HEMORRHAGE ON
VOLUME FLOW OF BLOOD THROUGH THE HIND PAW OF A NORMAL DOG

The influence of the vasomotor system on the relationship between blood pressure and blood flow is illustrated in Figures 5 and 6 In the sympathectomized dog, the flow varied directly with the pressure In the normal dog with intact vasomotor system wide variations in flow were encountered as the blood pressure was being reduced by hemorrhage After the blood pressure dropped below 70 mm Hg, the volume flow of blood remained constantly at a low level Although the flow in the sympathectomized dog was considerably reduced, it was still above that of the normal dog at this level of blood pressure

Analyses were made of the oxygen content and capacity and carbon dioxide content on the arterial and venous blood in one normal and one

TABLE II

Analyses of blood gases before and after hemorrhage in the normal dog in contrast to the sympathectomized dog

Dog number	Time	Arterial blood			Venous blood	
		Oxygen content	CO₂ content	Oxygen capacity	Oxygen content	CO₂ content
		volumes per cent	*volumes per cent*	*volumes per cent*	*volumes per cent*	*volumes per cent*
546 Normal control	Before hemorrhage	13 9	45 2	14 6	11 0	48.2
	Shock	14 5	15 1	15.3	4 6	23 9
14 Sympathectomized	Before hemorrhage	16 0	45 3	17 6	11 0	49.3
	After hemorrhage	14 5	31 8	15 2	4.5	41 1

sympathectomized dog at the beginning of the experiment and again at the end of five hours of low blood pressure   The results are given in Table II   After five hours of low blood pressure and diminished blood flow, the normal dog was in shock.   The elevated oxygen capacity of the arterial blood indicated the extent of hemoconcentration   In comparison the oxygen capacity of the sympathectomized dog was lower than at the start of the experiment.   The venous oxygen content in both dogs was reduced to the same extent, 4 5 volumes per cent, as a result of the diminished blood flow   Acidosis however, was present in the dog in shock   The arterial carbon dioxide was reduced from 45.2 to 15 1 volumes per cent.   In the sympathectomized dog, on the other hand, a comparable degree of acidosis was not present   The carbon dioxide was reduced only from 45 3 to 31 8 per cent.

DISCUSSION

The importance of an adequate supply of blood to the tissues of the body has been emphasized by many investigators in their studies on the mechanism of shock   Erlanger Gesell, Gasser and Elliott (11) concluded that the causative factor of shock was ' reduced circulation brought about possibly through the action of pain stimuli, and of a certain amount of hemorrhage, on the vasoconstrictor mechanism "   Gesell (12), after

his studies on the relationship of hemorrhage and "tissue abuse" to the blood flow through the salivary gland stated that the "volume flow of blood appeared to be the more fundamental problem "   During the World War, Bayliss (13) wrote "At the risk of tiresome iteration, I would again emphasize the importance of adequate oxygen supply to the tissues "   Johnson and Blalock (14) stressed the importance of a reduction in the output of the heart from a diminished blood volume as the centrally important feature in the development of shock   Since low blood pressure characterizes shock it was natural that attention should have been focused upon this aspect of the problem   Cannon (15) in his monograph on shock stated, "One of the central problems if not the most important central problem, of shock is that of discovering the reason for the lowered arterial pressure."   Porter (16) was the first to call attention to the "critical level " of blood pressure and Cannon (15), in his experiments with cardiac tamponade in cats, found that if the blood pressure were reduced for a period of time below the critical level, 70 mm Hg, a condition of shock ensued

Vasoconstriction, since it diminishes the capacity of the vascular bed and thus helps to maintain the blood pressure, has generally been regarded as a beneficial reaction   Through vasoconstriction however, at the same time that the pressure is maintained in the larger arteries, the "nutrient flow " through the smaller vessels is reduced

The data in the present experiments indeed show that vasoconstriction initially performs a beneficial function   The normal dogs were able to tolerate a greater loss of blood than were the sympathectomized dogs (Table I)   The blood pressure was not reduced to as great an extent even though more blood was lost.   The volume flow of blood in the periphery, on the other hand was reduced to a greater extent   Associated with this reduction in nutrient flow, hemoconcentration took place and the process of shock was initiated

From analysis of the relationship of blood pressure to blood flow in the absence of vasomotor impulses (Figure 5), it is clear that the head of pressure was of preeminent importance in regulating the volume flow   Superimposed upon this

control was that exercised by the vasomotor system (Figure 6)   In the normal dog, wide variations in flow were encountered during the period of initial bleeding over the higher ranges of blood pressure   Then, below 70 mm Hg, the flow was drastically and consistently reduced   We feel it to be of significance that the marked reduction in flow should occur at approximately 70 mm Hg, the so-called "critical level" (16)   At blood pressures below 70 mm Hg, although the blood flow through the paw of the sympathectomized dog was reduced, the tissues were probably still able to obtain sufficient blood for their needs   Vasoconstriction in the normal dog, at these pressures, still further reduced the blood supply and shock was produced.

Vasoconstriction after hemorrhage serves to protect the vital centers against the harmful effects of a dangerously low blood pressure   The effects of a period of prolonged low pressure on the heart were frequently observed   If blood were too rapidly reinjected after a period of low blood pressure, evidence of acute cardiac failure would supervene   The blood pressure would fall   Recovery could then be effected by withdrawing some of the blood and reinjecting it more slowly   In three of the experiments (Numbers 751, 767, and 2, Table I), the dogs were killed by too rapid reinjection of blood   Postmortem examination revealed a dilated right heart   The necrosis of the liver cells observed in the sympathectomized dog which was sacrificed (Figure 3, C) may have resulted from the prolonged low blood pressure, but our data are insufficient to warrant drawing conclusions

The brain is another vital center which is susceptible to injury from low blood pressure   In one of the sympathectomized dogs (385) and in one of the normal dogs which recovered (641), although the blood flow and blood pressure returned to normal after the reinjection of blood, the dogs showed definite signs of cerebral damage, manifested by stupor and extensor rigidity   This observation serves again to stress the useful function of the sympathetic nervous system   By means of vasoconstriction, preferential treatment of blood supply is given to the brain, since the cerebral vessels do not respond as vigorously to vasoconstrictor impulses as do the peripheral vessels (17)   The general tissues of the body are deprived of blood in order that the brain may live   After sympathectomy, this preference is lost   All the tissues of the body are accorded the same treatment   If the brain is receiving sufficient blood for its needs, all the tissues of the body are probably adequately supplied   As long as the brain is kept alive in the sympathectomized animal, the tissues will be kept alive also

Another observation which is in accord with the concept of an inadequate circulation as the fundamental cause of shock is offered by the determinations of the oxygen and carbon dioxide contents of the arterial and venous bloods   In the shocked dog (Table II), there was not only a severe reduction in the oxygen content of the venous blood as a result of the sluggish flow, but the carbon dioxide content was also reduced   This reduction in the carbon dioxide content, formerly believed to be of significance in the etiology of shock (Acapnea theory of Henderson (18)), is considered at the present time by most investigators to be of importance only in illustrating the acidosis from the accumulation of "fixed acids" which results from an inadequate circulation   In the sympathectomized dog, although the venous oxygen was reduced just as much as in the normal dog in shock, the "acidosis" was not as severe   Although the tissues of the dog which recovered were not receiving as abundant a supply of blood as normally, there was still sufficient oxygen to prevent as great an accumulation of fixed acids as in the dog which died

The volume flow of blood through the hind paw of the dog is not an exact index of the circulation through other portions of the body   Blalock and Levy (19) have recently presented evidence to show that in shock, the blood flow through the hind quarters of the dog is reduced to a greater extent than that through other regions   In our experiments it has served primarily to illustrate the reduction in circulation brought about by vasoconstriction in comparison to the diminution which resulted from the fall in blood pressure, since the flow through the normal paw was lower than the flow through the sympathectomized paw at comparable low levels of blood pressure   The values obtained for the

volume flow of blood through the paw in the shocked dogs are in general agreement with previous data obtained on the blood flow through the hand in clinical cases of surgical shock (20) In the present experiments upon normal dogs, when the blood flow was reduced to below 2 cc per minute, shock was produced In two normal dogs which recovered, the blood flow was 2 1 and 2.9 cc per minute. In two of the sympathectomized dogs, however, the blood flow through the paw was reduced to 1 0 cc per minute and yet recovery took place It seems likely that a minimum blood flow requirement exists, and if the nutritive flow is reduced below this level pathological changes ensue. Our data are insufficient to state precisely at what flow such changes occur

Recovery took place in two of the normal dogs In one of these dogs the blood pressure was kept between 50 and 80 mm Hg for two hours and in the other for six hours In both dogs, the flow was rarely reduced below 2 cc per minute. Both dogs showed dilution of the blood The dogs were calm and phlegmatic, and it was our impression that their lack of fear, in comparison with other normal dogs, prevented them from going into shock, even though the amount of blood lost was approximately the same.

One dog (Figure 4) Number 641, had been about the laboratory for several weeks and was well adjusted to experimental procedures This dog's blood pressure dropped abruptly to 80 after the first two hemorrhages and yet the blood flow varied between 12 and 19 cc per minute for an hour though the blood pressure ranged from 64 to 82 mm Hg In comparison, Dog 25 (Figure 1) was very high strung After the first two hemorrhages, the blood pressure rapidly built back to 100 mm Hg but the blood flow was reduced below 2 cc. per minute Over the next three hours although the blood pressure ranged between 76 and 96 mm Hg the blood flow stayed below 2 cc per minute In the 'well adjusted" dog rapid dilution of the blood took place since the hemoglobin fell from 103 to 75 per cent (16 5 to 11 4 grams per 100 cc.) in the course of two hours In the apprehensive dog, the hemoglobin during a two hour period fell only from 106 to 98 per cent (16 6 to 15.3 grams per 100 cc) It is well recognized that fear produces vasoconstriction and a reduction in peripheral blood flow (20)

It is probable that the reduction in blood flow, observed in Dog 25 even before the hemorrhage, was the result of emotional stimulation After hemorrhage although the arterial blood pressure was maintained at a higher level as a consequence of the activity of the sympathetic nervous system, the blood flow was so far reduced that it facilitated the process of shock. In Dog 641, the high preliminary level of blood flow before the hemorrhage indicated that the sympathetic nervous system was not being called into activity Even after hemorrhage, vasoconstriction was minimal The blood flow continued at a high level, dilution of the blood took place, and the dog recovered

Similarly, Dog 546 (Table I) recovered although the blood pressure was reduced to 66 for two and one-half hours At the second experiment on this same dog it was necessary to reduce the blood pressure to an average of 38 for almost four hours before shock was produced Even so the volume flow of blood through the paw was definitely higher (1 6 cc. per minute) than in the other dogs which went into shock

In a previous communication, the hypothesis was advanced that the process of shock has its origin in the physiological reactions of the body to traumatic stimuli By means of activity of the sympathetic nervous system, the body is able to adjust itself to the emergency If the crisis is too protracted, however, then vasoconstriction, the very mechanism by which the organism strives to survive, brings about its ultimate dissolution The present experiments on the effects of hemorrhage offer further support to this hypothesis

SUMMARY

A condition of shock was produced in normal dogs by means of hemorrhage (Figure 1) This condition was characterized by hemoconcentration, failure to respond to blood transfusion, and characteristic pathological changes in the tissues (Figure 3)

After total sympathectomy, even though the blood pressure was reduced to a lower level, for a longer period of time, shock was not produced (Figure 2) Dilution of the blood took place there was prompt and beneficial reaction to blood transfusion and similar pathological changes in the tissues did not occur (Figure 3)

The sympathectomized dogs, however, were unable to tolerate as large hemorrhages as the normal dogs The blood pressure also fell to a lower level at an earlier period than in the normal dogs (Table I)

The difference in reaction of normal and sympathectomized dogs to hemorrhage was correlated with the peripheral blood flow In the normal dog, as the blood pressure was reduced by hemorrhage to 70 mm Hg, the blood flow was reduced below 2 cc per minute (Figure 6) In the sympathectomized dog, at the same level of blood pressure, the blood flow was above 2 cc per minute (Figure 5)

In two normal dogs which recovered, although the blood pressure was reduced to between 60 and 80 mm Hg, the blood flow continued above 2 cc It was our impression that the absence of fear in these dogs predisposed them to recovery

Vasoconstriction in the presence of hemorrhage gives preferential treatment of blood supply to the vital centers, the heart and the brain In the sympathectomized dog, such preference is lost All the tissues of the body are accorded the same treatment As long as the vital centers receive sufficient blood supply, all the tissues of the body probably receive an adequate amount of circulation, and the condition of shock is prevented

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A SIMPLE METHOD FOR THE ESTIMATION OF TOTAL PROTEIN CONTENT OF PLASMA AND SERUM [1] I A FALLING DROP METHOD FOR THE DETERMINATION OF SPECIFIC GRAVITY

By BENJAMIN M KAGAN

(*From the Department of Medicine, School of Medicine Johns Hopkins University and Hospital Baltimore*)

In 1926, Barbour and Hamilton described a falling drop method for determining specific gravity (1) They used a mixture of xylene and bromobenzene in a tube 30 cm in length With the use of standard solutions and nomograms, they obtained an accuracy within 1×10^{-4} in specific gravity Guthrie and Kruse in their unpublished work, found that a mixture of methyl salicylate and mineral oil permitted the use of a shorter tube (2) The present method is an extension of their work

A mixture of methyl salicylate and mineral oil was chosen because such a mixture has a higher viscosity so the tube length can be shortened and a more uniform temperature obtained throughout the oil In addition, it is less volatile than the xylene-bromobenzene mixture (Methyl salicylate B P 222.2° C, mineral oil 190.6° C, m xylene 138.8° C, bromobenzene 156.2° C)

The ultimate aim was to obtain the specific gravity of blood serum at 25.0° C referred to water at 25.0° C., *i e*, 25°/25° This temperature was chosen because it was most convenient when the determinations were done at room temperature

THEORY

Stokes' law indicates that the rate of fall of a small solid sphere in a viscous fluid is a function of the radius and specific gravity of the sphere, the specific gravity and viscosity of the fluid, and the acceleration due to gravity This method provides that a drop of fluid may be timed as it falls through an oil with which it is not miscible The radius of the drops is kept constant by the use of a calibrated pipette to deliver a definite volume. The specific gravity and viscosity of the oil being

kept constant, the specific gravity of the drop may be determined from its rate of fall in the oil.

MATERIALS

A mixture of synthetic methyl salicylate and heavy California mineral oil is used The proportions vary according to the range which is desired For serum and plasma, we have found a mixture of specific gravity 1.0130, 25°/25° C. most useful

This mixture is placed in a glass tube 15 to 16 cm long, having a uniform inside diameter of 14 mm This tube is etched with two rings which are exactly 10 cm apart, the lower one being 15 mm above the bottom of the tube. The tube is held upright in a glass water jacket which is equipped with a stirring rod and a thermometer to read 0.1° C between 20° and 30° C This tube
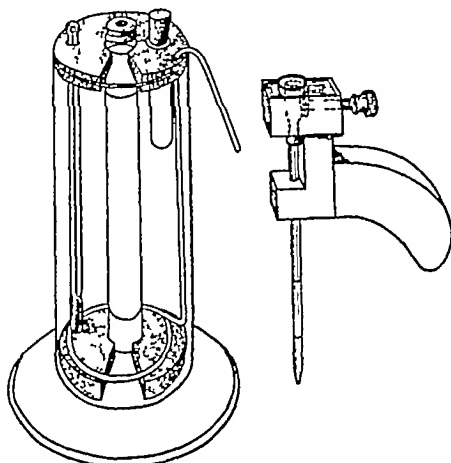
FIG. 1 APPARATUS FOR DETERMINATION OF SPECIFIC GRAVITY BY FALLING

is filled to 15 mm from the top with the oil and provided with a one-holed disc to center the pipette when the drop is released Within the water jacket, there is a small test tube in which the serum may obtain the same temperature as the oil (Figure 1)

The pipette is made from unconstricted capillary tubing calibrated to deliver 0 015 cc (15 c mm) between two marks A wooden block is provided which contains a rubber bulb and a metal screw The pipette fits into the bulb When the screw is turned, it presses upon or releases the bulb and this permits fluid to be drawn into or discharged accurately from the pipette

A stop watch which reads in tenths of a second is used

METHOD

The fluid to be tested is placed in the small test tube until it comes to the temperature of the water bath It is then drawn into the pipette to the level of the first ring, and the tip of the pipette is placed under the surface of the oil in the tube A drop of 15 c mm is delivered beneath the surface of the oil, and as the pipette is withdrawn, the drop remains in the oil and falls The time

required by the drop to pass between the two rings on the tube (10 cm) is determined with the stop watch

Calibration Standard solutions of sodium chloride are made so that they cover the range 1 0140 to 1 0370, 25°/25° as determined by pyknometry (4) A large pyknometer (about 65 cc) enables one more easily to obtain accuracy of the order of 10^{-5} Ten determinations of the falling time are then done on each solution and the average falling time plotted against the specific gravity of the standard as in Figure 2 The temperature must be kept at 25 0° C during the standardization Owing to difference in viscosity, particularly in various samples of mineral oil, slight differences in results are obtained unless each new preparation is standardized in this manner

Temperature correction factor As the temperature rises, the viscosity and specific gravity of the oil decrease The specific gravity of the fluid to be tested also decreases as the temperature rises If the temperature of the fluid to be tested is kept at the same temperature as the oil, all the variables can be accounted for at one time. In
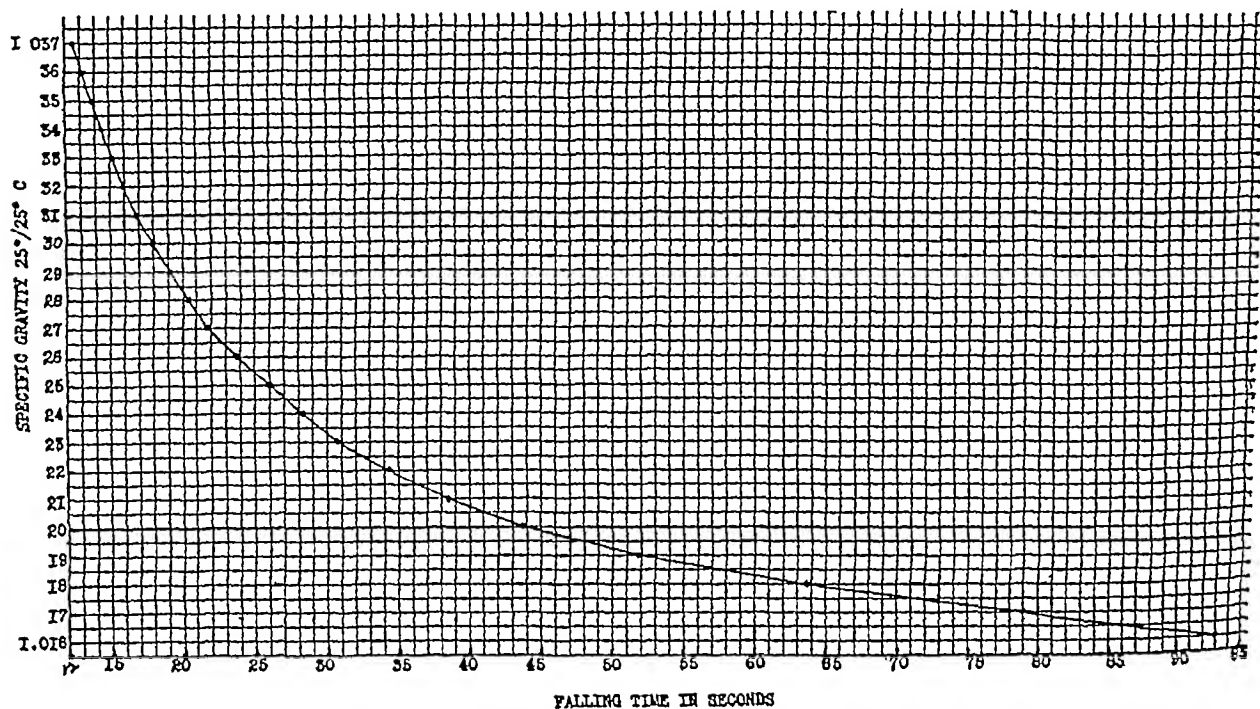
FIG 2. RELATION OF FALLING TIME TO SPECIFIC GRAVITY

Graph paper must be used which will permit the reading of tenths of a second and specific gravity to 1×10^{-4}
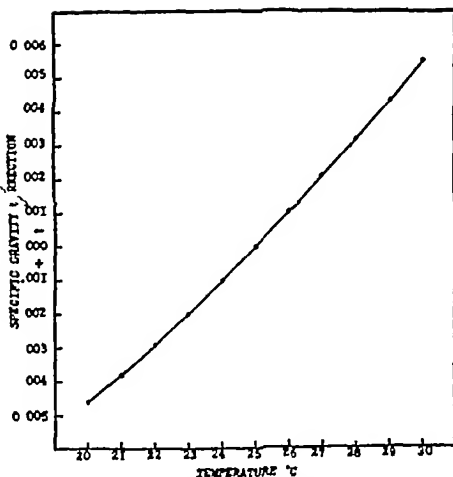
FIG 3   SPECIFIC GRAVITY CORRECTION

The ordinate gives the value which must be added (+) or subtracted (—) from the specific gravity read from Figure 2 for the falling time determined at any temperature represented in the abscissa.

Figure 3 is given the correction which must be made on the specific gravity as read from Figure 2 for the falling time at any temperature between 20° and 30° C   It is not necessary to reconstruct this chart for each new preparation of oil

Figure 4 illustrates the effect of temperature on the specific gravity of serum alone. The weight of pooled serum which filled a large pyknometer at various temperatures ($X°$ C) was determined and referred to the weight of the same volume of water at 25 0° C   The resultant specific gravity $X°/25°$ was plotted against the temperature at which the serum was weighed   Thus, it may be seen that the temperature of the serum need be only within 0.5° C. of that of the oil in order to obtain accuracy within 1×10^{-4} in specific gravity

Error of the single drop   In the application of this method, we have used an average of 3 determinations   The accuracy is, however, not appreciably affected if only one determination is done with care. Thus the timing of drops in the range of 13 to 30 seconds checks within 0.2 second   When drops take longer than 30 seconds to fall the variations in time of fall of individual drops is greater   At this point, however, the curve becomes more flattened   In this range, the difference in timing of separate drops does not mean as much in specific gravity so that the accuracy over the entire range of specific gravity remains about the same

Application of the method to human serum and plasma. The specific gravity of 28 specimens of human   serum   and   oxalated   and   heparinized
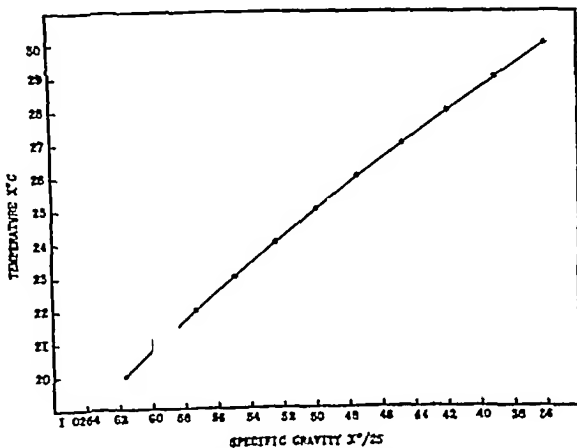
FIG. 4   EFFECT OF TEMPERATURE ON THE SPECIFIC GRAVITY OF Σ

TABLE I

*A comparison between the specific gravity of serum and
plasma as obtained by use of a 2 cc pyknometer
and the drop method*

Material	Specific gravity		Difference
	Drop method	2 cc pyknometer	
Serum	1 0285	1 0288	−0 0003
	1 0256	1 0254	+0 0002
	1 0222	1 0222	0 0000
	1 0281	1 0283	−0 0002
	1 0283	1 0283	0 0000
	1 0306	1 0304	+0 0002
	1 0280	1 0280	0 0000
	1 0286	1 0290	−0 0004
	1 0280	1 0283	−0 0003
	1 0289	1 0292	−0 0003
	1 0303	1 0302	+0 0001
	1 0283	1 0279	+0 0004
	1 0209	1 0210	−0 0001
	1 0277	1 0279	−0 0002
	1 0285	1 0286	−0 0001
	1 0278	1 0274	+0 0004
	1 0297	1 0295	+0 0002
	1 0220	1 0220	0 0000
	1 0287	1 0284	+0 0003
	1 0261	1 0262	−0 0001
Oxalated plasma			
	1 0234	1 0233	+0 0001
	1 0310	1 0312	−0 0002
	1 0268	1 0268	0 0000
	1 0330	1 0328	+0 0002
	1 0234	1 0232	+0 0002
Heparinized plasma			
	1 0272	1 0274	−0 0002
	1 0297	1 0298	−0 0001
	1 0221	1 0223	−0 0002

plasma was determined by the use of a 2 cc pyknometer according to the method described by Moore and Van Slyke (3) A comparison of the results obtained by the two methods is shown in Table I The greatest difference was 4×10^{-4} The falling time was taken at various temperatures and the correction factor used as indicated above

SUMMARY

A simple, rapid, and easy method for the determination of specific gravity is presented which is based upon Stokes' law for the velocity of falling bodies The materials and methods are described in detail In ordinary usage, the method will yield the specific gravity of serum or plasma with a maximum difference from that determined by a 2 cc pyknometer of 4×10^{-4} and has a range of 1 0150 to 1 0370

This work was begun as a result of the author's association with Dr Roy R. Snowden in his laboratory in Pittsburgh Particular indebtedness is due Professor Wm Mansfield Clark for his invaluable advice. The author is also grateful to Dr T K. Kruse of Pittsburgh Medical School for his cooperation

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A SIMPLE METHOD FOR THE ESTIMATION OF TOTAL PROTEIN CONTENT OF PLASMA AND SERUM. II. THE ESTIMATION OF TOTAL PROTEIN CONTENT OF HUMAN PLASMA AND SERUM BY THE USE OF THE FALLING DROP METHOD

By BENJAMIN M. KAGAN

(From the Department of Medicine School of Medicine Johns Hopkins University and Hospital Baltimore)

An instrument for the accurate estimation of serum protein which has the simplicity of a hemoglobinometer has an obvious place in medicine Peters and Van Slyke (1) have reviewed the many physical methods which have been tried and conclude that the specific gravity is the most accurate. They find the colorimetric method liable to errors up to 10 per cent Moore and Van Slyke (2) found the relationship between the specific gravity and protein content of human heparinized plasma could be expressed by the equation of a straight line

$$P = 343(G - 1\,0070)$$

in which P represents grams of protein per 100 cc. and G the specific gravity They found the maximum deviation from the value of total protein estimated by the gasometric method to be 0 6 gram per cent. Zozaya (3) found that the above formula did not give good results with human serum Weech *et al* (4) used dog's blood and demonstrated a linear relationship for serum and for heparinized plasma. They used the micro-Kjeldahl as standard and claimed an even better correlation than Moore and Van Slyke. In all of these investigations, the specific gravity was determined by the use of pyknometers and delicate balances Moore and Van Slyke found the falling drop method of Barbour and Hamilton (5) for specific gravity to give as good results as pyknometry, but because the method required the constant use of standards and nomograms, they gave it up An analysis of the falling drop method which is used in this investigation and a comparison with that of Barbour and Hamilton is given in the first section of this paper (12)

METHODS

The specimens of blood were obtained by venepuncture from patients in the hospital and out-patient departments Most of the patients were selected because they were known to have abnormalities in their blood proteins or in other blood constituents Total serum protein was determined by the macro Kjeldahl method using the methyl red methylene blue indicator as described by Johnson and Green (6) Refractometric readings were done routinely The results obtained by the latter method are better than those generally reported because an as yet unpublished correction factor is used which is based upon thousands of macro Kjeldahl determinations The specific gravity was determined by the falling drop method and represents the weight of serum at 25 0° C referred to the weight of the same volume of water at the same temperature The correlation between total protein and specific gravity was determined by the method of least squares (7)

The chloride content was determined by the method of Van Slyke (1) and the cholesterol content by the method of Sackett (8) [1]

RESULTS

Serum The results on 107 specimens of sera are plotted in Figure 1 The linear relationship may be expressed by the equation

$$P = 345(G - 1\,0076),$$

where P is the total grams of protein per 100 cc. of serum and G the specific gravity 25°/25° C The mean deviation of total protein as estimated from this formula and that determined by macro-Kjeldahl was ± 0 16 gram per cent The greatest deviation was + 0 48 In the same sera, the mean deviation of the total protein value deter-
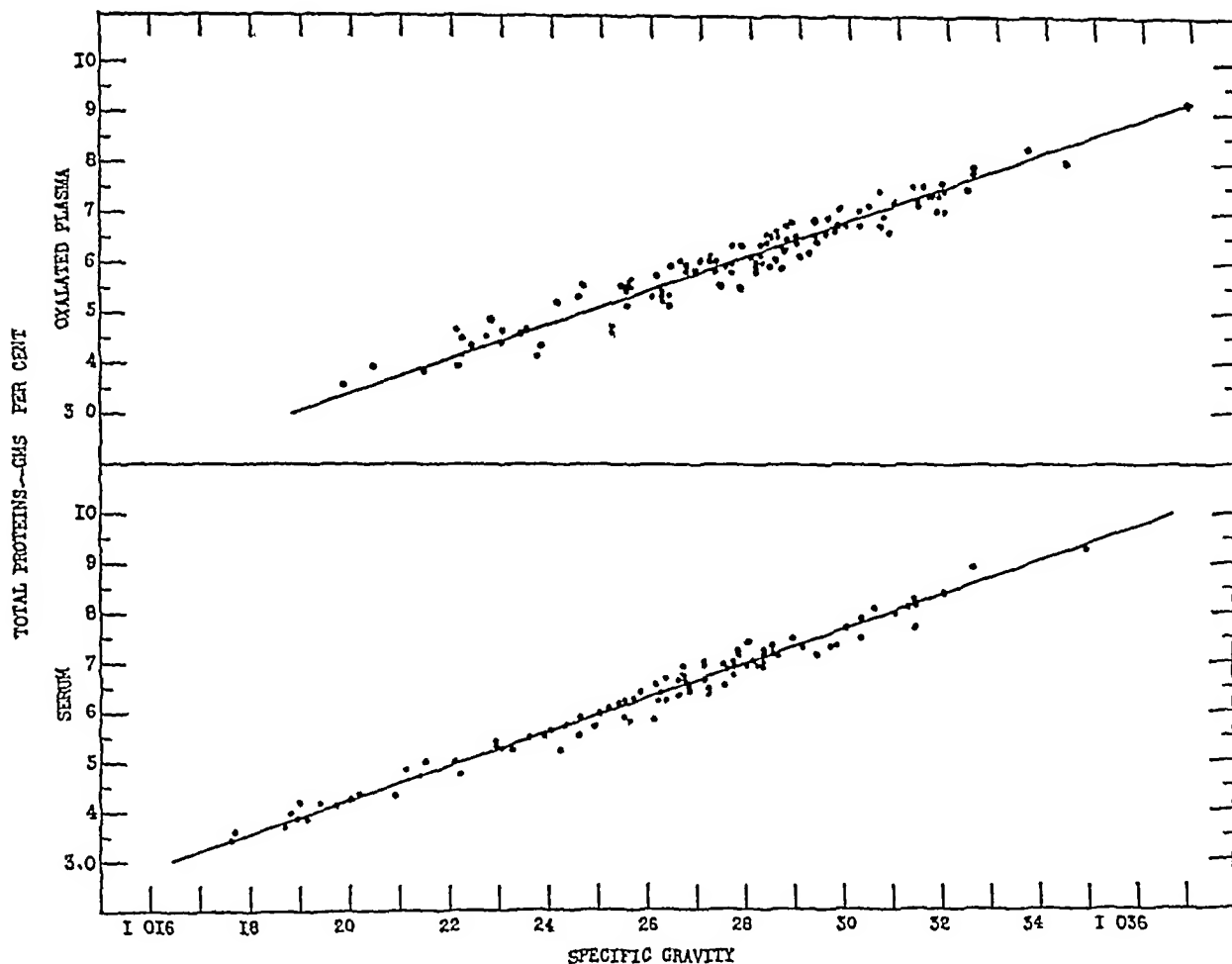
FIG 1   RELATION OF SPECIFIC GRAVITY TO TOTAL PROTEIN CONTENT OF PLASMA AND SERUM

mined by refractive index and that by Kjeldahl was ± 0 37 and the greatest deviation — 1 04 gram   In over 25 per cent of the specimens, the deviation of the estimation of refractive index from the Kjeldahl was more than 0 45 gram per cent

Plasma   The plasma was prepared by drying 5 drops of 10 per cent potassium oxalate in bottles to which 10 cc of blood was added   Such an amount of oxalate added to distilled water raises its specific gravity 0 0023, 25°/25° C   The results on 122 specimens of plasma are also plotted in Figure 1   The relationship may be expressed by the equation

$$P = 340(G - 1\ 0099)$$

The mean deviation of total protein as calculated from this formula and that determined by macro-Kjeldahl was ± 0 23   The greatest devia-

tion was + 0 59   In the same plasma, the mean deviation of total protein estimated by refractometry and that by Kjeldahl was ± 0 36   The greatest deviation was + 1 50 grams per cent In 10 specimens, the difference was greater than 0 6 gram, and in 3 greater than 1 00 gram

The estimation of total protein in plasma is not as accurate as in serum, but 10 cc of blood were not always added to the same amount of oxalate and this probably accounts in large part for the difference   In addition, oxalate is known to withdraw a variable amount of water from the cells and so dilute the plasma

The effect of variations in the A/G ratio

On 79 of the total 229 specimens, the A/G ratio was also determined   They varied from 34/66 to 76/24, but it was not possible to find any correlation between the ratio and the deviations of the

total protein as estimated by Kjeldahl nitrogen determinations and specific gravity This might have been expected, since Nugent and Towle (9) separated albumin from globulin and found their effect on specific gravity to be the same

The effect of variations in the nonprotein solids

Since the protein content of serum is between 75 and 80 per cent of the dry weight, a close correlation with specific gravity is to be expected The remainder of the total solid content is composed of a number of constituents each of which is measured in milligrams per 100 cc The most important of these with regard to specific gravity and protein correlation appear to be fat and cholesterol We have had 10 specimens in which the cholesterol values were known to be over 200 mgm per cent The results are shown in Table I

TABLE I

Effect of variations in cholesterol content

Specimen	Cholesterol	Total protein		Devia tion
		Kjeldahl method	Falling drop method	
	mgm per cent	*grams per cent*	*grams per cent*	*grams per cent*
Serum	289	4 19	4 20	+0 01
	315	6 98	7 18	+0 20
	358	6.82	6.98	+0 13
	664	4 82	5 01	+0 19
	812	4 76	5 17	+0 41
Plasma	266	6.57	6 87	+0.30
	289	4 19	4 05	−0 14
	315	6 77	6 85	+0 08
	333	6 77	7 15	+0.38
	789	4 85	5 36	+0.51

It does not appear that moderate elevations in cholesterol content affect the usefulness of the method Most specimens with high cholesterol also have high fat content and this probably counterbalances the effect of cholesterol on specific gravity

On 29 specimens, the chloride content ranged from 84 4 to 108 6 m eq , but no correlation with the deviation between the specific gravity and Kjeldahl estimates of protein was apparent.

A comparison of serum with oxalated plasma and heparinized plasma

Blood drawn from 3 patients was used in this experiment. From blood which was taken at one

TABLE II

A comparison of serum with heparinized and oxalated plasma

Sub-ject	Specimen	Formula: Total protein =	Specific gravity	Total protein		
				Specific gravity	Kjeldahl	Refractive Index
				grams per cent	*grams per cent*	*grams per cent*
M T	Serum	345(G−1.0076)	1.0220	4 97	4.66	4.00
	Oxalated plasma	340(G−1.0099)	1.0234	4.59	4.63	4.00
	Heparinized plasma	343(G−1.0070)	1.0221	5 18	4.82	4.00
H H	Serum		1.0261	6.38	6 11	6.30
	Oxalated plasma		1.0268	5 75	6.06	5.90
	Heparinized plasma		1.0272	6 93	6.71	6.30
G J	Serum		1.0287	7.28	7.21	6 90
	Oxalated plasma		1.0310	7 17	7 12	6 90
	Heparinized plasma		1.0297	7 79	7.56	7.00

venepuncture into a single syringe, serum, heparinized plasma, and oxalated plasma were prepared The total protein was estimated by Kjeldahl, specific gravity, and refractometry The specific gravity was determined in all cases by the falling drop method The total protein of heparinized plasma was estimated from the specific gravity by the formula of Moore and Van Slyke (2) In all three cases, the oxalated specimens contained the lowest protein content and the heparinized plasma the highest. In one instance, the total protein content of the oxalated specimen differed by 0 65 gram per cent *according to the macro-Kjeldahl method* Heparinized plasma contained an average of 0 4 gram per cent more protein than serum because it contains fibrinogen The low values with oxalate are probably due to the withdrawal of water from the cells

DISCUSSION

Rowe (10) and others have found that stasis during the withdrawal of blood from the patient, with pressure intermediate between venous and arterial, causes in one minute an increase of 0 17 gram per 100 cc. in the protein content of plasma due to loss of water from the blood vessels In ordinary clinical routine, no distinction is made between values obtained from plasma or serum yet it has been shown that oxalated plasma may contain 0 3 to 0 4 gram per cent less protein than serum (11) and heparinized plasma may contain as much as 0 65 gram per cent more protein than oxalated plasma No matter what method is used

to estimate the protein content of a specimen, its accuracy with regard to clinical application will depend for the greater part on the manner in which it is collected and on the way it is prepared

SUMMARY

A new method for the estimation of total serum or plasma protein is presented It is based upon the linear relationship which exists between the specific gravity and the protein content The specific gravity is determined by a new falling drop method which is easy, time saving, and can be done with extremely small quantities of blood It provides a measure of the protein content with an accuracy which exceeds clinical requirements, and is about twice as accurate as the refractometric method

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THE CHEMICAL COMPOSITION OF VOLUNTARY MUSCLE IN MUSCLE DISEASE A COMPARISON OF PROGRESSIVE MUSCULAR DYSTROPHY WITH OTHER DISEASES TOGETHER WITH A STUDY OF EFFECTS OF GLYCINE [1] AND CREATINE THERAPY [2]

By JOHN G REINHOLD AND GEORGE R. KINGSLEY

(From the Division of Biochemistry The Laboratories Philadelphia General Hospital and the Department of Physiological Chemistry Schools of Medicine University of Pennsylvania Philadelphia)

Current interest in disease of the muscles has emphasized the need for information concerning chemical changes within affected muscle. For this reason we have analyzed during the past four years specimens of muscles of patients suffering from primary progressive muscular dystrophy and other diseases involving the musculature. Aside from the desirability of securing data for theoretical purposes, it was our object also to learn whether changes occurring in these diseases were sufficiently characteristic to be applied for differential diagnostic or therapeutic purposes Our studies were planned in conjunction with therapeutic trials of glycine and other agents in muscular dystrophy When possible, specimens were secured before, during, and after treatment for the purpose of ascertaining effects upon the composition of muscle Our observations have been discussed, in part, in an earlier report (17) dealing with treatment of progressive muscular dystrophy, although publication of chemical data was deferred pending investigation of additional patients The present paper describes the results of chemical examinations made upon patients whose histories were included in the report mentioned as well as others studied subsequently

EXPERIMENTAL

This report includes results of examination of muscle removed at biopsy from 12 patients suffering from progressive muscular dystrophy and from 16 patients with other diseases involving

muscles primarily or secondarily Eight of the patients treated for progressive muscular dystrophy by administration of glycine or creatine submitted to additional biopsies after receiving these substances for varying periods Except where other muscles are mentioned, specimens were removed from the medial portion of the *vastus externus*

Specimens were excised rapidly and prepared for analysis as described previously (17) The trichloroacetic acid extract of muscle, prepared at 0° C., was analysed according to the procedure of Eggleton and Eggleton (9) for total acid soluble phosphorus, phosphocreatine (barium soluble fraction hydrolysed rapidly by acid at 20° C), soluble ester phosphorus (the remainder of the barium soluble fraction after subtracting phosphocreatine), inorganic phosphate (directly determined barium insoluble fraction), and adenosine triphosphate (barium insoluble phosphate liberated by 7 minute hydrolysis at 100° C. in normal acid) When it was not possible to prepare extracts of muscle in the cold, analyses of labile phosphate compounds were omitted Phosphate was determined by the method of Fiske and Subbarow (10) and creatine by the method of Folin (11) When sufficient material was available, determinations of fat, nitrogen, and water were included Results are expressed in terms of fat-free muscle when the concentration of fat was known

Clinical and necropsy findings (available in 3 cases) and results of microscopic examination of muscle were given consideration in establishing diagnoses Protocols and salient details of microscopy of 8 patients have been described in the earlier article cited Histological studies of muscle were made by Dr R. P Custer

RESULTS

Chemical analyses of specimens of muscle of patients with progressive muscular dystrophy are shown in Table I Data in this table are arranged according to the severity of the disease interpreted in terms of ability to use the voluntary muscles

In the early stages of muscular dystrophy, despite the absence of marked functional impairment, creatine concentrations already were lowered Further loss of creatine and of other constituents known to participate actively in muscle metabolism occurred as the disease advanced In the late stages, exceptionally low concentrations were found Infiltration of fat explained much of the loss, however, when allowance was made *for this factor,* concentrations remained below those similarly calculated for normal muscle Solids other than fat were decreased in many specimens, at times considerably Replacement of contractile by fibro-areolar tissue, an outstanding change in many specimens, likewise acted to diminish concentrations of extractives Estimates based on the amount of connective tissue shown by examination under the microscope suggested that this factor also could not account for changes in extractives A definite conclusion is not possible *until quantitative measurement of connective tissue is accomplished*

Total acid soluble phosphorus of primary dystrophic muscle usually was lowered, roughly in proportion to the diminution of creatine However, several specimens contained acid soluble phosphorus in concentrations within or near normal limits in the presence of low concentrations of creatine Possibly, phosphorus from calcified necrotic areas may have contributed to bring about such disproportionately high values In normal muscle the concentration of creatine was 2 5 to 3 times that of total acid soluble phosphorus As shown in Table I, this ratio is frequently lowered in progressive muscular dystrophy, and for a time this fact was regarded as evidence of creatine deficiency in this disease Subsequently, similar values were found, not uncommonly, in secondary disease of the muscles

Creatine combined as phosphocreatine showed declines from the normal that often exceeded the change in total creatine This was true particularly when the disease was advanced At this stage approximately one-half of the creatine was present as phosphocreatine as compared with two-thirds so combined in earlier stages

In addition to the decrease in concentration of phosphorus combined with creatine, phosphorus measured as adenosine triphosphate (or adenyl pyrophosphate) was diminished in comparison

TABLE I

Composition of voluntary muscle in progressive muscular dystrophy

Date	Patient†	Age	Duration	Muscle creatine	Total acid soluble	Phospho- creatine	Soluble ester	Inor ganic	Adenosine triphosphate	Total nitrogen	Water	Ether extract	Micro- scopic
		years	*years*	*mgm per 100 grams of fat free muscle*						*grams per 100 grams of fat free muscle*	*grams per 100 grams of muscle*		*grade**
Apr 15, 1934	Rutl	17	9	162	145								B
May 17, 1933	H J (7)	12	½	219	109	32	25	19	31	2 88	82 0	21 5	B
May 17, 1933	T J (8)	13	1	161	99	25	18	17	26	3 39	79 5	23 5	B
Mar 5, 1935	Morro	17	2	139	74								B
Jan 10, 1933	R M (3)	27	7	388	276	86	56	44	89	2 86	81 8	61 3	B
Jan 31, 1933	H M (6)	10	2	150	52	12	8	16	14	3 03	81 1	19 3	C
Jan 31, 1933	J M (5)	11	4	87	53	11	11	16	17	1 74	88 1	26 0	C
Jan 31, 1933	A S (4)	8	5	37	24	6	2	9	7	3 64	77 4	40 0	D
July 8, 1932	L G (2)	45	15	55	14	6		5		1 42	90 8	27 0	D
June 2, 1932	H M (1)	28	19	99	37	12	4	10	10	1 81	89 0	71 6	D
Jan 21, 1937	Pars	18	10	116	52						75 0		C
June 1, 1934	Roger	30	20	186	64					2 73	82 8	44 0	C

* B = slight deterioration   C = marked deterioration   D = severe deterioration
† Numbers in parentheses refer to case numbers of preceding article (17)

with normal concentrations Inorganic and soluble ester phosphorus, although decreased in concentration along with other fractions, constituted a larger proportion of the total acid soluble phosphorus in dystrophic muscle than in normal muscle This relative gain was at the expense of phosphocreatine phosphate and adenosine triphosphate.

Degeneration of muscle did not occur uniformly in progressive muscular dystrophy Not only were certain groups of muscles affected before and with greater severity than others, but variation occurred within the muscle as well Fibers in various stages of degeneration were seen together with normal fibers Larger areas of muscle differed in concentration of creatine, phosphorus, and fat. Table II shows the composition

TABLE II

Composition of specimens removed 20 hours postmortem from different portions of right and left vastus externi (Progressive muscular dystrophy)

	Creatine	Acid soluble phosphorus	Total nitrogen	Water	Ether extract
	mgm. per 100 grams of fat free muscle		*grams per 100 grams of fat free muscle*		*per cent*
Right					
Distal	166	67	2 66	80 1	60 9
Medial	225	95	2 69	77 0	47.5
Proximal	183	63	2.30	80 4	42 6
Left					
Medial	142	52	2 84	77 9	27.2

of specimens removed postmortem from scattered areas of the same muscle and of the corresponding muscle of the opposite leg of a patient who had suffered many years from progressive muscular dystrophy Portions of muscle that differed widely in appearance were selected purposely so as to ascertain the extent of variation in chemical composition The differences observed considerably exceeded those encountered in the course of multiple biopsies from a restricted area or from patients in whom the disease had not reached a terminal stage

The clinical effects of glycine therapy upon these patients have been described in our preceding article (17) which included also a brief summary of alterations occurring within the muscles The examination of the muscles of patients suffering from progressive muscular dystrophy after varying periods of glycine feeding (up to

1 year) showed that decided abnormalities, both chemical and histological, persisted However, higher concentrations of extractives found in muscle suggested that the glycine fed had stimulated regeneration of muscle Unfortunately, only 8 patients could be induced to submit to serial biopsies and while 6 showed significant gains in chemical composition of muscle following treatment, this number is insufficient to exclude chance variation Such improvement, if it may be so considered, was not manifested clinically despite the fact that glycine was administered for as long as 23 months to two patients Similar trials with creatine showed no favorable effects The composition of muscle remained unchanged or exhibited additional evidence of deterioration The distribution of acid soluble phosphorus remained unaffected by either glycine or creatine therapy

Analyses of muscle similar to those made in progressive muscular dystrophy have been made in other diseases involving the musculature to learn whether changes observed were peculiar to dystrophy or of more general occurrence (Table III) With the exception of one patient who had myasthenia gravis, and one with myositis, involvement of the muscles was caused either by impaired metabolism or by disease primarily of the nervous system.

In myasthenia gravis (Patient 33), the only distinctly abnormal result was the exceptionally high concentration of soluble ester phosphorus Nevin (16) also found this to be the sole noteworthy chemical change in muscle in this disease Likewise, Collazo, Barbudo, and Torres (5) observed no distinctive deviation from normal in muscle of a patient suffering from severe myasthenia gravis In Table III, it is seen that the concentration of creatine is not far from normal and not nearly as low as in progressive muscular dystrophy While Wilhams and Dyke (19) reported lowered creatine concentrations in muscle in myasthenia gravis the method employed fails to measure creatine quantitatively

Osteitis fibrosa cystica (secondary to parathyroid adenoma) in Patient 34 whose muscles we examined was accompanied by painful sensations and pronounced weakness in the voluntary muscles. Large amounts of creatine were excreted in the urine Muscle creatine and phosphocreatine

TABLE III   PART I

Composition of voluntary muscle in diseases other than muscular dystrophy, including muscles normal in appearance

Patient number	Date	Patient	Sex	Age	Diagnosis	Duration	Functional Impairment	Biopsy
				years		*years*		
19	Oct 2, 1934	Towk	M		Questionable trichinosis		Weakness of legs	Deltoid, normal
20	April 22, 1934	And	M	27	Trichinosis suspected, unconfirmed		None	Normal
21	July 10, 1937	Rich	F		Hypertensive cardiovascular disease		None	Normal
22	Feb 21, 1934	Vanc	M	49	Progressive atrophy secondary to degeneration of central nervous system of circulatory origin	10+	Slight	Atrophied fibers
23	Feb 21, 1934	Baer	M	40	Muscular atrophy	5	Moderate	Resembled muscular dystrophy
24	Feb 21, 1934	Vedd	M	42	Progressive muscular atrophy secondary to degeneration of central nervous system of circulatory origin	7	Moderate	Slight atrophy
25	Oct 2, 1934	Lewi	M	68	Progressive atrophy	30	Moderate	Severe atrophy
26	Feb 3, 1936	Famb	M	32	Multiple sclerosis	7	Moderate	Hypertrophy
27	Feb 15, 1937	Di Fi	M	43	Progressive spinal muscular atrophy		Moderate	Focal atrophy, minor degeneration
28	Dec 11, 1935	Sco	M		Undetermined		Moderate	
29	Jan 23, 1937	McLa	M	28	Muscular atrophy, postinfluenzal	6	Moderate	Atrophy
30 a b c	Aug 21, 1935	Lohr	M	35	Landry's ascending paralysis of unknown etiology	1/12	Moderate	Vastus externus Proximal Distal Deltoid
31	Oct 29, 1936	Tara	M		Amyotrophic lateral sclerosis		Marked	Atrophy, degeneration
32	Dec 21, 1935	Cande	M	44	Amyotrophic lateral sclerosis	1/2	Marked, total areflexia	Simulated progressive muscular dystrophy
33	Feb 3, 1934	Engel	F	33	Myasthenia gravis	2	Marked	Atrophy
34	Feb 3, 1934	Raim	M	45	Hyperparathyroidism, osteitis fibrosa cystica	1	Moderate	
35	April 27, 1933	Stan	F	38	Disuse atrophy, inanition	2	Moderate	Atrophy
36	April 27, 1933	Tayl	F	63	Diffuse myositis	1/4	Marked	Chronic inflammation

vere lowered The proportion of total acid soluble phosphorus present as inorganic phosphorus was increased

The effects of semi-starvation for months upon the composition of muscle is illustrated by Patient 35 (Stan) Weakness and atrophy were outstanding symptoms, and striking improvement followed forced feeding While both creatine and total acid soluble phosphorus were lowered, there was no significant change in the distribution of phosphorus

Changes in muscle occurring in myositis were discussed in our earlier paper Losses of creatine and total acid soluble phosphorus are comparable with those in dystrophy (Patient 36) In contrast to the latter, the distribution of acid soluble phosphorus differed less from normal

Impairment of the muscles, obviously secon-

dary to diseases of the nervous system, existed in 10 patients (Patients 22 to 27, and 29 to 32) This group, like the others already discussed, included various grades of involvement of the muscles Compared with the results in muscular dystrophy, chemical changes were not as pronounced, although the duration of the disease was equal to or in excess of that of the dystrophic patients The distribution of phosphate did not differ significantly from normal It is apparent from Table III that in most patients the concentration of creatine had been sustained near normal levels or had not diminished greatly Exceptionally low values were found only in two, both diagnosed amyotrophic lateral sclerosis In the latter, replacement of muscle fibers by fat and fibro-areolar tissue was widespread, and the resemblance to dystrophic muscle was close

PART II

No	Urine		Muscle*								
	Preformed creatinine	Creatine as creatinine	Creatine	Phosphorus					Total nitrogen	Water	Ether extract
				Total acid soluble	Phospho-creatine	Soluble ester	Inorganic	Adenosine triphosphate			
	grams per 24 hours			*mgm per 100 grams of muscle*					*grams per 100 grams of muscle*		
19			449	164							
20			296	122	50	12	14	40			
21			435	175							
22	0 230	0.389	*482*	*137*	*72*	*8*	*6*	*33*	*3 14*	*72 6*	*8 9*
23			*372*	*181*					*2 66*	*71 0*	*9 0*
24	0 169	0 419	*350*	*171*					*1 88*	*77 1*	*8 9*
25	0.217	0 107	*321*	*126*	*52*	*17*	*20*	*35*	*2 55*	*76 5*	*7.5*
26			269	134							
27			263	117							
28			217	124							
29			209								
30a			161	133							
b		↲	151								
c			136	125						78 7	
31			119	91							
32			89(R)†	53(R)†							
			86(L)†	57(L)†							
33	0.829	0.383	*320*	*154*	*54*	*40*	*8*	*45*	*2 94*	*75 8*	*3 7*
34	0 504	0.336	260	134	30		24	31			
35	0 201	0 183	258	85	39	5	22	17	*2 04*	*74 7*	10 9
36	0.304	0.253	*175*	*85*	*33*	*9*	22	24			

* When italicized values are calculated on fat free basis.
† (R) = Right
  (L) = Left

Specimens from muscle normal in function and appearance were secured from Patients 19 and 20 suspected of trichinosis, but in whom neither parasites nor inflammation of muscles were found, and also from a patient suffering from hypertensive cardiovascular disease   Creatine concentrations were within the limits of normal established by Bodansky (1) and Corsaro (6) in two patients, and lower in the third   Few figures are available for concentrations in human voluntary muscle of phosphocreatine and other fractions of the acid soluble phosphate. Nevin (16) has published analyses for these substances in 6 normal human subjects   Our values for phosphocreatine and inorganic orthophosphate agree well with those of Nevin, however, we find less adenosine

triphosphate and more soluble ester phosphorus Our data are similar to those of Eggleton (8), Milroy (15) and others who have analysed animal muscles

DISCUSSION

The effect of disease upon the composition of muscle has not been investigated extensively Brand and Harris (4), Nevin (16), Collazo, Barbudo, and Torres (5), and Debrè, Marie, and Nachmansohn (7) have analysed phosphate compounds of the muscles of patients suffering from myopathies   The last named group determined glycogen and lactic acid as well   Low concentrations of creatine in muscle were found in myositis fibrosa by Bodansky Schwab, and Brindley (2)

and in myositis ossificans by Bodansky and Schwab (3) Nevin (16) has compared a number of diseases in regard to muscle chemistry Hines and Knowlton (12) have studied quantitatively the rate of loss of muscle extractives that follows denervation, while Chor, Dolkart, and Davenport (20) have correlated histological and chemical changes in muscles of cats and monkeys after denervation

Our studies show that changes in composition of voluntary muscles were more pronounced in progressive muscular dystrophy than in most of the other diseases investigated including myasthenia gravis, secondary involvement of muscle due to disease of the central nervous system, or certain disturbances of endocrine origin Changes comparable to those observed in progressive muscular dystrophy were encountered in polymyositis and in amyotrophic lateral sclerosis

Our experience suggests that chemical analysis can be used advantageously in the study of muscle involvement It seems probable that analysis of muscle can supplement microscopy for quantitative evaluation of impairment and that it perhaps is capable of supplying information not otherwise procurable Although many constituents of muscle are affected, determinations of phosphate, creatine, and possibly glycogen are the more practicable, while analyses of fat and water are desirable According to data now available, low concentrations of extractives denote deterioration of muscle tissue On the other hand, poor function associated with comparatively normal chemical composition appears to characterize diseases in which the defect is primarily one of transmission of impulses However, it is evident that there is no sharp differentiation between the so-called myopathies and neuropathies Amyotrophic lateral sclerosis, for example, showed loss of creatine from the muscle comparable to that in progressive muscular dystrophy, yet the former is regarded by most authorities as belonging to the group of neuropathies

Although differing appreciably from normal, the distribution of acid soluble phosphorus in the badly deteriorated muscle of advanced dystrophy was not changed to the extent anticipated on the basis of morphological alteration Inhibition of chemical reactions of the muscles, including those involved in hydrolysis of phosphate compounds

provides an explanation That hydrolysis following stimulation does not occur as readily in diseased as in normal human muscle is shown by Nevin's data (16) In his experiments, no decrease in adenosine triphosphate and little change in phosphocreatine occurred after stimulation of badly degenerated muscle Muscles less severely involved showed an appreciable hydrolysis of phosphate compounds as did also normal muscle.

Presumably, deficiencies with respect to phosphocreatine and adenosine triphosphate were related since phosphorylation in muscle involves both in linked reactions Interference at any of several steps in the phosphorylation process would lead to impairment of phosphocreatine resynthesis with diminished concentration of this substance Loss of diffusion of creatine remaining uncombined would follow Actually, we have found less creatine combined as phosphocreatine in most of the dystrophic muscles when comparison is made with muscles in other diseases studied

Through the collaboration of Doctor William A Wolff and Professor D Wright Wilson, several specimens of biopsied muscle from patients with progressive muscular dystrophy were analysed by the colorimetric method for carnosine (13, 14) It was found that carnosine was present although in low concentrations as compared with the normal The decrease was equivalent to that exhibited by other acid-soluble extractives in the same specimens of muscle.

SUMMARY

1 The chemical composition of muscle in progressive muscular dystrophy was altered more extensively than in diseases with secondary atrophy of the muscles Changes comparable to those found in progressive muscular dystrophy were observed in diffuse myositis and amyotrophic lateral sclerosis

2 In progressive muscular dystrophy, concentrations of creatine and other substances extractible by dilute acid were diminished Phosphocreatine and adenosine triphosphate constituted a smaller proportion and soluble ester phosphorus and inorganic phosphorus a larger proportion of the total acid soluble phosphorus compared with control specimens of muscle normal in appearance

3 Chemical analysis of muscle can be used to

supplement clinical and histological examination in diagnosis and in measurement of deterioration of muscle

We are indebted to Doctors J W McConnell, George Wilson Joseph C. Yaskin, Bernard Alpers, and their assistants of the Department of Nervous Diseases Philadelphia General Hospital for clinical observations and diagnoses.

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THE LATE EFFECTS OF BILATERAL CAROTID SINUS DENERVATION IN MAN

REPORT OF TWO CASES WITH STUDIES OF THE VASCULAR REFLEXES

BY RICHARD B CAPPS AND GÉZA DE TAKÁTS

(From The Circulatory Group St Luke s Hospital Chicago)

Owing principally to the investigations of Hering (1) and Heymans *et al* (2), it is now well established that the carotid sinus and the aortic depressor nerve and its end organs are the chief nervous mechanisms for the regulation of the blood pressure. Considering their importance from a physiological viewpoint, it should not be surprising if surgical interference produced untoward effects In animals, denervation of both carotid sinuses and section of both depressor nerves has been found by most investigators (1, 3, 4, 5) to cause a chronic hypertension and tachycardia, an increased lability of the blood pressure and pulse, and often various other disorders Denervation of the carotid sinuses alone usually results in similar but only temporary changes (1,4) For example, in 8 out of 10 dogs on whom Leriche *et al* (6) had performed this operation, the blood pressure and pulse returned to normal in less than two months However, in the 2 remaining animals there was still an elevation of the blood pressure of 20 and 40 mm Hg respectively at the end of this time Regarding other effects of this operation, especially the effect on postural vascular reflexes, there are very few data in the literature. Hering (1) states that, in dogs, there occurs a greater fall in blood pressure on assuming the standing position than in normal animals Green and his coworkers (7) noted fainting attacks in 2 of their dogs who had bilaterally denervated carotid sinuses These attacks may have been on a postural basis

In man unilateral denervation of the carotid sinus results in only temporary elevation of the blood pressure and pulse, and, as far as is known, no change in the postural vascular reflexes (6 8) Bilateral carotid sinus denervation in man has not previously been reported in this country In the European literature there are several reports, but the published data are very meager Lauwers (9) found no serious effects in 9 cases He includes no actual figures for blood pressures or pulse rates

Danielopolu (10) merely reports that the operation has been done and that no hypertension resulted Five cases were operated upon by Leriche *et al* (6) who state that no marked permanent variations in blood pressure or pulse rate occurred However, figures are given for only 3 of the cases In one of these the pressure returned to the preoperative level the day of operation, in another there was a hypertension 3 days postoperatively (further observations were not recorded), and in the third case the blood pressure was still elevated at the end of 4 months from a preoperative level of 140/70 to 165/85 In none of the above cases were observations on the effect of posture on the blood pressure recorded

From this review of the literature, it is apparent that our knowledge of the late effects of bilateral carotid sinus denervation in man is scanty and incomplete. Only resting blood pressures and pulse values have been determined, and then only in a few cases We were fortunate in having available 2 patients on whom one of us (Géza de Takáts) had performed bilateral carotid sinus denervation 8½ and 17 months previously Since both patients were operated upon for epilepsy, complete preoperative data are not available. In spite of this deficiency, a study of the vascular reflexes of these 2 patients at this relatively late date postoperatively has yielded results of sufficient interest to warrant the present report

METHODS OF STUDY

The observations made were directed at determining (1) the presence or absence of expected abnormalities in vascular reflexes (2) the response of the carotid sinuses to stimulation and (3) the status of another vasovagal reflex, the oculocardiac. Identical tests were made on both patients and in most cases each test was repeated at least once, and in cases of doubt several times. Where

marked variations occurred on repetition, the most extreme results have both been recorded Blood pressures were determined by the auscultatory method Pulse rates were taken at the wrist, being calculated from consecutive 15-second intervals for the postural and exercise tests For the other tests an electrocardiograph was used

In the first category were included observations on the resting blood pressure and pulse, and the changes that occurred on voluntary standing and after exercise Resting observations were obtained after 15 minutes or more in the supine position. Postural changes were determined by having the patient stand up quickly with as little effort as possible The blood pressure was taken each 15 seconds and the radial pulse rate counted continuously, while the pulse was calculated for each 15-second interval The findings at the end of 2 minutes are recorded in the tables, since a reasonably constant level seemed to be reached by this time. The effect of exercise was determined by allowing the patients to run up and down one flight of stairs The maximum change in blood pressure and pulse rate, taken at 15-second intervals as before, is recorded in Table I

In the second category were included observations on the effect of direct carotid sinus pressure and of occlusion of the common carotid low in the neck with sudden release of pressure. The patients were tested in the supine position The technique has been described in a previous paper (8) Continuous electrocardiographic tracings were made and the pulse calculated therefrom

TABLE I

Effect of bilateral denervation of the carotid sinus on blood pressure and pulse

Patient	Age	Sex	Preoperative lying		Months post operative	Postoperative lying	
			Blood pressure	Pulse rate		Blood pressure	Pulse rate
	years		*mm Hg*	*per minute*		*mm Hg*	*per minute*
M	30	M	102/72	70	17	102/68	58
S	23	M	120/60	70	8½	122/70	72

Determinations of blood pressure were made as frequently as feasible during the experiment. The maximum changes in pulse (time between two beats) and in blood pressure have been recorded Changes in respiration and subjective symptoms were also noted The effects of strong eyeball pressure for a 15-second interval were studied in a similar manner

MATERIAL

The 2 patients studied were both cases of severe idiopathic epilepsy The physical examination and the laboratory findings were essentially negative. The operations were performed by one of us (Géza de Takáts), and consisted of a 2-stage denervation of the carotid sinuses and a 2-stage cervicodorsal sympathectomy The

carotid sinuses were cleanly stripped, as well as the artery, for a distance of 2 cm., both above and below the sinus (A more complete description of the operation, as well as the effect on the epilepsy, will be discussed in a later publication) The carotid body was identified at biopsy in one instance Many autonomic nerve fibers were found in all four biopsy specimens

As controls for the postural tests, the results obtained by Roth (11) in 37 normal males and in 5 cases of bilateral cervicodorsal sympathectomy were used. Her determinations were made with the same technique as was used here. For the exercise test, 7 normal subjects—5 females and 2 males recruited from the doctors and nurses of the hospital staff—were employed as controls

RESULTS

In Table I is recorded the resting blood pressure and pulse of both patients before the operation and at the time of study, along with other pertinent data It will be noted that there was practically no change in the blood pressure The significance of the slower pulse in Patient M postoperatively is problematical

TABLE II

Effect of bilateral denervation of the carotid sinus on postural vascular reflexes and reaction to exercise

Patient	Lying		Standing		Standing after exercise		Symptoms on standing
	Blood pressure	Pulse rate	Blood pressure	Pulse rate	Blood pressure	Pulse rate	
	mm Hg	*per minute*	*mm. Hg*	*per minute*	*mm. Hg*	*per minute*	
M	107/68	58	85/64	80	95/65	100	None
S	122/70	72	106/78 or 75/60	91	103/70	114	Temporary faintness. Fainted once

Controls	Average change						
37 Normal males (Roth (11))	Sys. −1 9 Dias. +9 5	+15 8					
5 Cases of bilateral cervicodorsal sympathectomy (Roth (11))	Sys. +0 8 Dias +10.8	+11					
7 Normal subjects					Sys. +20* Dias. −17	+31	None

* In the seven normal subjects the average changes in blood pressure and pulse rate are computed from a control level while standing at rest

Table II shows the effect of posture and of exercise In both patients it will be noted that there is a marked fall in the systolic blood pressure on assuming the standing position In both the normal control group and in those with a bilateral

cervicothoracic sympathectomy, there was no significant change in the systolic pressure. The diastolic pressures in the 2 patients failed to rise in the same manner as in the controls The fall in blood pressure of Patient S was found to vary considerably The extremes of this variation have both been included in Table II In the second instance a pressure of 58/45 was maintained for a full minute before it gradually rose to 75/60 where it remained Strangely enough, the pulse changes were greater in extent than in the controls

After running up one flight of stairs, the patients failed to show either a pulse or a blood pressure rise as great as that in the normal controls

Finally, it will be noted that Patient S usually felt faint immediately after rising from the lying position On one occasion he was observed to lose consciousness and fall He stated definitely that this experience was a faint and not an epileptic attack. Furthermore, he said that before the operation he had not had these symptoms

TABLE III

Vascular reflexes after bilateral denervation of the carotid sinus Patient M

Test	Control		During pressure		On release		Hyperpnoea	Symptoms
	Blood pressure	Pulse rate	Blood pressure	Pulse rate	Blood pressure	Pulse rate		
	mm. Hg	per min-ute	mm. Hg	per min-ute	mm. Hg	per min-ute		
Right carotid sinus pressure	110/72	103	102/68	100			0	None
Left carotid sinus pressure	140/78	88	134/7	94			0	Coughed. Test unsatisfactory
Right carotid occlusion.	110/68	77	120/7	85	106/7	81	0	None
Left carotid occlusion.	118/76	83	123/7	85	118/7	85	0	None
Pressure on right eyeball	100/84	77	100/84	73			0	None
Pressure on left eyeball	100/84	79	100/84	71			0	None

In Tables III and IV are shown the effect of carotid sinus pressure in each of the 2 patients It will be noted that in Patient M there was no definite response to any of the tests (Table III) The rise in blood pressure, with occlusion of the common carotid, might be so considered, but the lack of a depressor response on the release of pressure is in favor of some other explanation than a reflex from the carotid sinus

TABLE IV

Vascular reflexes after bilateral denervation of the carotid sinus Patient S

	Control		During pressure		On release		Hyperpnoea	Symptoms
	Blood pressure	Pulse rate	Blood pressure	Pulse rate	Blood pressure	Pulse rate		
	mm. Hg	per min-ute	mm. Hg	per min-ute	mm. Hg	per min-ute		
Right carotid sinus pressure	122/78	83	105/7	63			0	Very slight dizziness
Left carotid sinus pressure	130/82	85	100/80	72			+	Very slight dizziness
Right carotid occlusion.	120/78	75	118/78	75	118/78	75	0	None
Left carotid occlusion.	120/76	81	124/78	78	120/80	77	0	None
Pressure on right eyeball	116/74	65	104/72	62			+	None
Pressure on left eyeball	120/78	73	106/7	62			+	Felt slightly dyspneic

In Patient S (Table IV) there was a slight but definite response to direct stimulation of either carotid sinus Occlusion of the common carotid failed, however, to induce any definite changes Pressure on the eyeball in this patient caused a slight but definite fall in blood pressure and some slowing of the heart.

DISCUSSION

From the foregoing results it can be concluded that no permanent hypertension or tachycardia resulted from bilateral carotid sinus denervation. Furthermore, at the time of examination there was no evidence of increased sympathetic activity or increased lability of the blood pressure and pulse as might be demonstrated by exercise. However, on the other hand, there was a definite postural hypotension Several questions concerning the validity of these conclusions immediately come to mind

One first wonders whether the denervations were complete or whether regeneration of the nerves may not have occurred This, of course, cannot be answered categorically The actual operation performed was an extensive one that should result in a complete denervation Temporary hypertension was observed following the second operation in both cases This lasted for at least 12 days in Patient M and for at least 4 days in Patient S Because of the patients' discharge on these days, the duration of

tension is not known Patient M also had a mild increase in pressure of 20 mm Hg after his first sinus denervation This lasted for 7 days, at which time the second operation was performed The hypertension in each case was of a considerable degree, reaching 152/100 mm Hg in Patient S and 146/100 in Patient M The production of hypertension lasting a number of days is evidence that most if not all of the carotid sinus fibers were sectioned Further evidence that the sinuses were completely denervated is offered by the results of carotid sinus stimulation Both direct mechanical stimulation of the sinuses and indirect stimulation by occluding the common carotid arteries gave no definite evidence of any physiological activity It is true that in Patient S there was a mild response to direct pressure on both sides This type of response has, however, been noted before in the absence of physiological activity (8) The explanation is not clear Possibly the nerve stumps are sensitive (2) The presence of reflex changes from pressure on the eyeball simply demonstrates that other vasovagal reflexes are sensitive in Patient S The next question that arises is that of the influence of the cervicodorsal sympathectomies The 5 patients studied by Roth (11) and included as controls in Table II show that this operation does not effect the reaction to change in posture On theoretical grounds, there should not be any effect on blood pressure reflexes Furthermore, it is known that sympathetic accelerator fibers reach the heart through ganglia much lower than the second dorsal (12) which was the lowest one resected in our patients In fairness, it must be said that the depressed acceleration of the pulse in the exercise test may have been owing, at least in part, to the section of the chief sympathetic nerve supply to the heart

The absence of proper preoperative controls throws doubt only on the finding of postural hypotension However, it seems unlikely that both patients would have shown this same phenomenon preoperatively and especially to such a marked degree The absence of postural symptoms in Patient S before the operation is good evidence that they were the result of the denervation

The significance of the more direct tests of the carotid sinuses is, of course, questionable because of lack of adequate preoperative control studies The negative results, however, are consistent with

complete denervation and absence of nervous regeneration

The fact that bilateral denervation of the carotid sinus in man fails to produce a permanent elevation or an increased lability of the blood pressure or pulse shows that the aortic depressor mechanism is able to assume these functions of the carotid sinus in a reasonably satisfactory manner Possibly also, as Breucker (13) has suggested, the type of specialized nerve endings found in the carotid sinus are more extensively distributed than generally thought The postural hypotension can be explained on the basis of a relatively lower sensitivity of the aortic depressor mechanism than of that of the carotid sinus Koch (14) has adduced experimental evidence in animals in favor of this assumption

Whether or not certain cases of idiopathic postural hypotension are due to a mechanism similar to that of our two cases cannot be stated Certainly, direct stimulation of the carotid sinus in such cases frequently reveals an absence of sensitivity (15, 16) It may well be that for various reasons the physiological sensitivity is diminished in certain individuals

In conclusion, it should be emphasized that until further cases have been studied, the operation of bilateral carotid sinus denervation should not be undertaken lightly It may be followed by unfavorable and even serious after-effects It should not be performed unless the symptoms and condition of the patient justify the risk

CONCLUSIONS

1 Two cases have been reported where a bilateral carotid sinus denervation and a bilateral cervicodorsal sympathectomy were performed The vascular reflexes of these patients were studied 17 and 8½ months after the operations respectively

2 There was no elevation of the blood pressure or pulse rate at this time as a result of the operation

3 There was no increased lability of the blood pressure and the pulse as shown by an exercise tolerance test

4 A marked postural hypotension was found in both patients

5 Evidence that the foregoing findings were significant is presented

6 The possibility that a similar mechanism, namely loss of sensitivity of the carotid sinuses to normal physiological stimuli, may account for the findings in certain cases of idiopathic postural hypotension is pointed out.

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THE RENAL FACTOR IN ARTERIAL HYPERTENSION WITH COARCTATION OF THE AORTA [1]

By DAVID A RYTAND

(From the Department of Medicine Stanford University School of Medicine, San Francisco)

Consideration of the hydrodynamics in coarctation of the aorta indicates that the arterial hypertension which is a feature of that disease is not to be explained upon the grounds of mechanical obstruction to blood flow *per se* Such consideration has led to experiments which show that this variety of hypertension is owing to interference with *renal* blood supply

Pathological physiology of coarctation of the aorta In this condition, arterial pressure is practically always high in the arms, while it is usually low or normal (occasionally even high) in the legs (1) The hypertension in the upper part of the body is often attributed to the mechanical obstruction of the stenotic aortic isthmus to blood flow, but as Lewis (2) pointed out, the total cross section area of the collateral bed is at least equal to that of the normal aorta. However, the collateral vessels are long and tortuous and may easily account for the reduced pressure in the lower body as compared with the upper Although the resistance of the collateral bed explains the difference between arm and leg pressure, it is not an explanation of the absolute pressure levels, were mechanical obstruction the only factor, one would always expect to find low pressure in the legs and less increase of pressure in the arms than actually occurs

If the increased arterial pressure in the arms were a result of redistribution of blood such as occurred in the experiments of Barcroft and Samaan (3) then the cardiac output and flow of blood through the upper part of the body should be increased, this, however, has not been found (2, 4, 5, 6, 7) Failure to demonstrate abnormally high rates of flow can mean only that peripheral resistance is increased in all the small vessels of the upper part of the body, the increased resistance in this region requires just as much explanation as does the generalized in

creased resistance in any other variety of hypertension.

The presence of an increased resistance in the upper part of the body in coarctation of the aorta may perhaps be made clearer by a mathematical demonstration using the well known formula

$$\text{Resistance} = \frac{\text{Pressure}}{\text{Flow}}$$

With pressure in mm Hg and flow in liters per minute, resistance may be expressed in arbitrary units with less confusion than when dynes are introduced (8)

When the regional fractionation of the cardiac output of a normal man weighing 70 kgm and with a blood pressure of 125/80 mm Hg is calculated from the data of Levy and Blalock (9) for the dog and with mean pressure equal to diastolic pressure plus 43 per cent of pulse pressure (10), the representative results noted in Table I are obtained

TABLE I

Calculated (approximate) regional blood flow and resistance for normal man

Region	Blood flow	Resistance in arbitrary units
	liters per minute	$\left(\frac{100\ mm\ Hg}{liters\ per\ minute} \right)$
Upper trunk, arms, head neck, and heart	2 00	50 (A)
Kidneys	1 00	100 (B)
Liver and portal bed	1.50	67 (C)
Lower trunk and legs	0 65	154 (D)
Entire body (excluding lungs)	5 15	19 (E)*

* The resistance for the entire body (E) may also be calculated from A, B C and D by the formula $\frac{1}{E} = \frac{1}{A} + \frac{1}{B} + \frac{1}{C} + \frac{1}{D}$ for calculating the total resistance offered by several resistances in parallel.

If now we consider a patient with coarctation of the aorta whose cardiac output is normal (6, 7) whose regional blood flow is normal or very nearly so (2, 4 5, 11), and ᴜɪ is 195/100 mm Hg in the
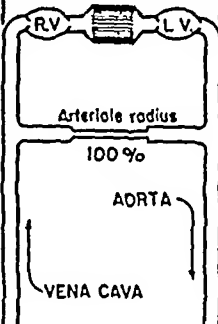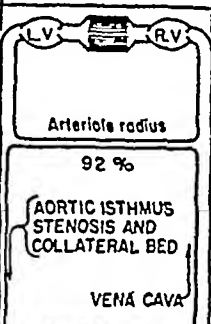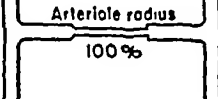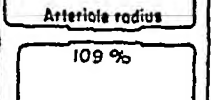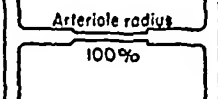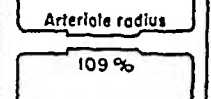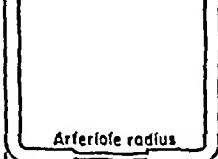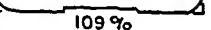
NORMAL	Resistance Arbitrary Units	Pressure Gradient (mm. Hg)	Blood Flow (Liters/minute)	REGION	Blood Flow (Liters/minute)	Pressure Gradient (mm Hg)	Resistance Arbitrary Units	COARCTATION OF THE AORTA
R.V. — L.V. Arteriole radius 100% AORTA VENA CAVA	50	100	2 0	Lungs — Upper trunk, arms, heart head and neck.	2.0	140	70	L.V. — R.V. Arteriole radius 92% AORTIC ISTHMUS STENOSIS AND COLLATERAL BED VENA CAVA
				Great Vessels	3.15	70	22	
Arteriole radius 100%	100	100	1 0	Kidneys	1 0	70	70	Arteriole radius 109%
Arteriole radius 100%	67	100	1 5	Liver and Portal	1 5	70	47	Arteriole radius 109%
Arteriole radius 100%	154	100	0 65	Lower trunk and legs	0 65	70	108	Arteriole radius 109%

FIG 1   REGIONAL RESISTANCE, BLOOD FLOW, ARTERIAL PRESSURE AND RELATIVE ARTERIOLAR RADIUS IN A NORMAL SUBJECT (BLOOD PRESSURE 125/80 MM HG) AND IN A SUBJECT WITH COARCTATION OF THE AORTA (BLOOD PRESSURE, ARMS 195/100, LEGS 80/60 MM HG)

Note. The combined caliber of aortic isthmus stenosis and collateral bed approximates that of the normal aorta   Its width is narrow in the diagram to indicate the resistance due to length and tortuosity

Hg in the legs, we may make the following calculations   Since the mean pressure in the lower aorta is approximately 70 mm Hg and that above the stenosis of the isthmus is 140 mm Hg, the resistance of the stenosis and collateral bed is represented by the pressure gradient (140 — 70 = 70 mm Hg) divided by the blood flow which they carry (abdominal viscera, lower trunk, and legs, or, 3 15 liters per minute), or 22 arbitrary units   As the tissues just mentioned are perfused with a normal volume flow per minute (2, 11) at a reduced pressure head, the resistance in this region must be decreased by 30 per cent   If the resistance had not decreased and the pressure had fallen from 125/80 to 80/60 mm Hg, then the flow would have been 30 per cent less than the observed level   But in the upper trunk, arms, head, neck, and heart, 2 0 liters per minute still circulate under a pressure head of 140 mm Hg, so that here the resistance must have increased

from 50 to 70 arbitrary units or by 40 per cent   Figure 1 shows these changes, the fundamental significance of which is not altered by the probable inaccuracies in the calculated absolute values of the regional flows

Since the resistance varies inversely with the fourth power of (arteriolar) radius, a decrease in radius of 10 per cent increases the resistance 50 per cent, and a decrease in radius of 15 per cent doubles the resistance   In coarctation of the aorta the resistance of the upper part of the body usually varies between 150 and 200 per cent of that in the lower part, so the radius of an arteriole from the arm would be only 15 per cent smaller than that of one from the leg   Bearing in mind the difficulties inherent in measuring fixed arterioles, one could have predicted that no significant difference in caliber of such vessels from arm and leg would be observed, a theoretical conclusion compatible with actual measurement (12)   In

this particular series, the calculated expected difference was only 10 per cent.

It seemed that the cause of the increased resistance and pressure in the upper body in coarctation of the aorta might be the same as that of the generalized changes in the Goldblatt dog (13), for in each case renal tissue is distal to a partially occluded artery It has been shown that the kidney is apparently unique in the pathogenesis of hypertension when an organ's artery is partially occluded (13) Bell's suggestion that the hypertension in coarctation of the aorta is fundamentally similar to that in the dog with a partially occluded renal artery was rejected by Goldblatt (14), [2] the problem has now been studied experimentally

Hypertension as evidenced by left ventricular cardiac hypertrophy was produced in 11 rats by partial occlusion of the aorta between the right (proximal) and left (distal) renal arteries With similar occlusion hypertension did not occur in 25 other rats when the left kidney was removed simultaneously In other words, *partial occlusion of the aorta at the same site in two groups of rats produced hypertension only in that group with renal tissue distal to the occlusion,* even though the blood flow was hindered by the same mechanical obstruction owing to stenosis and a collateral bed in both groups Hypertension was also produced in 12 rats by partial occlusion of the aorta just above both kidneys, a site more nearly analogous to that of the obstruction in coarctation of the aorta.

METHODS

The results in this paper were obtained from over 175 experiments on albino rats, each sacrificed 20 days after operation. The general conditions and methods used in this laboratory have been reported previously, the diet contained 10 per cent casein and 19 per cent total protein (15)

Operative technique The operative technique is a modification of Collins method (16) No attempt at asepsis is made. The rat is anesthetized with ether and a long midline abdominal incision made. If the left renal artery is to be tied, it is dissected free, usually be-

tween the kidney and the suprarenal vein occasionally it is best freed between the latter and the aorta. A wire 0 4 mm. in diameter is placed parallel to the exposed artery and a silk thread passed beneath both wire and artery The thread is then tied, the ligature of course including wire and artery The wire may then be gently withdrawn, the ligature remaining, the abdominal incision is closed in the usual manner If the aorta is to be tied, it may be freed at the desired site (between the renal arteries or just above them) and tied together with a wire 0.9 mm. in diameter Occasionally the thoracic duct is ruptured, but heals spontaneously The sizes of the wires given above are satisfactory for rats of about 100 to 200 grams, but practice is necessary to learn the appropriate force with which to tie the ligature. In a successful experiment, the kidney blanches on tying then undergoes reactive hyperemia immediately on removal of the wire.

Organ weights In rats sacrificed 20 days after operation, the heart and kidneys were weighed on torsion balances and compared with the predicted weights of those organs in normal rats of the same weight and sex. The predictions were based on the following formulae derived by Dr T Addis from data collected in this laboratory on over 1500 rats

Male heart	$HW = 12.6BW^{0.72} + 8.$
Female heart	$HW = 12.56BW^{0.73} + 1.5$
Male kidney (single)	$KW = 15 0BW^{0.72} + 10.3$
Female kidney (single)	$KW = 20.18BW^{0.64} + 6.1$

In these formulae, more accurate than the similar formulae based upon surface area ($kBW^{0.67}$), organ weights are in milligrams, body weights in grams. Statistical studies show that the normal variability of heart weight is so small that its coefficient of variation at a given range of body weight of 10 grams is only 3.7 per cent. This means that an observed deviation (from the weight predicted by the equations) of ± 10 per cent occurs only once in 143 normal rats, a deviation of ± 15 per cent occurs once in every 15 770 rats any individual deviation of ± 12 per cent or more is "almost certainly" significant. Kidney weight is normally more variable, its coefficient of variation being 9.8 per cent. Therefore, an observed deviation in kidney weight of ± 10 per cent occurs as often as once in every 3 rats ± 15 per cent once in 8, ± 25 per cent once in every 143 in order to be statistically significant, a single observed kidney weight must deviate ± 28 per cent from the prediction. Averages of groups of hearts or kidneys are much less variable. The rats in the present experiment were under exactly the same conditions as were those from which the data were obtained for the formulae (same diet, constant temperature, etc.) and the formulae are known to apply only under constant conditions.

The degree of cardiac hypertrophy was used as an index of the effect of the procedures on blood pressure. In the absence of anemia or metabolic disease when an increased L/R ratio is present, increased heart weight seems to be a better clue to the mean daily level of arterial pressure than

[2] Goldblatt and Kahn (J A M A, 1938 110 49 footnote 1 (h) and personal communication) find in the dog that partial occlusion of the aorta below both renal arteries is not followed by hypertension similar occlusion immediately above both renal arteries produces hypertension.

under anesthesia by methods which are, in our hands, not satisfactory Anemia was excluded by the fact that hematocrit values in a group of hypertensive rats averaged 93 per cent of those of controls The hearts from 23 nonhypertensive and 14 unselected hypertensive rats were fixed, and the right and left ventricles separated and weighed The L/R ratio, corrected as previously described (15), was 21 per cent greater in the hypertensive group, a difference five times its probable error and therefore statistically significant.

In order to translate the observed cardiac hypertrophy into blood pressure levels, it may be noted that Chanutin and Barksdale (17) have shown that in the rat a hypertrophy of 25 per cent occurs with a chronic hypertension of 160 to 170 mm Hg, 50 per cent with 180 mm Hg or over Since the present experiments were of shorter duration, the existing pressure levels were probably higher at any given degree of cardiac hypertrophy

RESULTS

Detailed protocols are given in the Tables, a summary is presented in Figure 2 The experiments are best discussed in six divisions

EXPERIMENT		ORGAN WEIGHTS, % DEVIATION FROM NORMAL			
RIGHT	LEFT	HEART	KIDNEY		NUMBER OF RATS
			RIGHT	LEFT	
A		+2%	+38%	-100%	14
		±0%	+39%	-75%	3
B		-2%	+34%	-100%	25
		±0%	+28%	-76%	1
C		+28%	+39%	-65%	9
D		+26%	+37%	-49%	7
E		+29%	-100%	+16%	4
F		+20%	-1%	-4%	12

Fig 2 Summary of Results Obtained in Rats Sacrificed 20 Days after the Operative Procedure Indicated by the Diagrams

A Partial occlusion of the left renal artery, with left nephrectomy This combined procedure was of course a control These 14 rats were not expected to have hypertension and in fact did not The average heart weight was + 2 per cent of

that predicted, while the right kidney presented the usual degree of compensatory hypertrophy (+ 38 per cent) In addition, three more rats (operated upon as in Experiment C) are included because in them the ligature was too tight, as a result the left kidney became necrotic (autonephrectomy)

B Partial occlusion of the aorta between the renal arteries, with left (distal) nephrectomy If the hypertension of coarctation of the aorta is to be explicable upon the basis of mechanical obstruction, these rats should have hypertension On the contrary, the average heart weight of 25 rats was 2 per cent less than predicted, and not a single rat had cardiac hypertrophy A possible objection is that the ligatures may have been too loose, the answers are (*a*) that these rats were operated upon alternately with those of Experiment D, in which hypertension occurred in one-third of the rats, and (*b*) in 6 rats of this series the aorta became *completely* obstructed (pulsations entirely absent) by intimal proliferation (Figure 3 shows the collateral circulation in such a rat) To these 25 rats may be added 1 from Experiment D in which the left kidney was completely necrotic

C Partial occlusion of the left renal artery This series was designed to demonstrate that a modification of the Goldblatt technique would produce hypertension in the rat That it does in fact do so is shown by the cardiac hypertrophy in 9 animals, as much as + 54 per cent and averaging + 28 per cent In these animals the left kidney atrophied (— 65 per cent) with little or no necrosis

D Partial occlusion of the aorta between the renal arteries In this experiment 7 rats had hypertension as evidenced by cardiac hypertrophy averaging + 26 per cent (as high as + 37 per cent), and in these the left (distal) kidney atrophied (— 49 per cent) just as in Experiment C

This is a series of rats operated upon alternately with those of Experiment B and *at exactly the same site, hypertension occurred only when there was renal tissue distal to the obstruction,* and only when this tissue was atrophic but not necrotic

E Partial occlusion of the aorta between the renal arteries, with right (proximal) nephrectomy So far in these studies hypertension has been uni-

FIG 3 ROENTGENOGRAM AFTER INTRACARDIAC INJEC
TION OF OPAQUE MATERIAL SHOWING THE COLLATERAL
CIRCULATION IN A NONHYPERTENSIVE RAT (LEFT
NEPHRECTOMY COMPLETE OCCLUSION OF AORTA DISTAL
TO RIGHT KIDNEY)

In Experiment C there were 6 rats and in D 16 rats in which neither cardiac hypertrophy nor left renal atrophy occurred presumably, the ligatures in these instances were too loose to alter hydrodynamics appreciably Likewise there were 23 rats in Experiment E and 7 in F without cardiac hypertrophy, the same explanation probably holds Such animals are therefore omitted from consideration since there is no evidence direct or indirect of aortic or renal artery stenosis Since they serve as controls their pertinent data are included in Tables IV to VII but averaged separately In another 51 rats in which the ligature was too tight necrosis of tissues beyond it occurred in a few days, when this occurred with the tie on the left renal artery the kidney became necrotic with the aorta tied the animal died

The concentration of urea in the blood is normal in coarctation. In the rats of Experiment F it varied from 30 6 to 38 9 mgm per cent, well within normal limits In those experiments in which unilateral nephrectomy was done, the blood

TABLE II

Experiment 1   Partial occlusion of the left renal artery
with left nephrectomy

	Weight of heart	Weight of kidney	
		Right kidney	Left kidney

TABLE III

Experiment B   Partial occlusion of the aorta between the renal arteries, with left (distal) nephrectomy

Sex	Body weight	Weight of heart			Weight of kidneys				
		Pre-dicted	Ob-served	Devia tion of observed from pre-dicted	Pre-dicted	Right Kidney		Left kidney	
						Ob-served	Devia tion of observed from pre-dicted	Ob-served	Devia tion of observed from pre-dicted
	grams	mgm	mgm	per cent	mgm	mgm	per cent	mgm	per cent
M	166	590	628	+ 6	596	744	+25		
M	170	600	630	+ 5	606	954	+57		
F	143*	521	545	+ 5	509	732	+44		
F	146	529	552	+ 4	516	672	+30		
M	164	585	608	+ 4	591	834	+41		
M	180	626	646	+ 3	631	842	+33		
M	153	556	572	+ 3	563	786	+40		
M	184	637	645	+ 1	641	908	+41		
M	164*	585	582	± 0	591	872	+48		
M	138	515	517	± 0	523	694	+33		
M	184	637	636	± 0	641	980	+53		
M	184	637	634	± 0	641	870	+36		
M	164	585	580	− 1	591	848	+43		
M	140	521	514	− 1	528	660	+25		
M	170*	600	587	− 2	606	846	+40		
M	188	648	632	− 2	651	894	+37		
F	108	422	412	− 2	425	537	+26		
M	290*	894	860	− 4	885	1020	+15		
F	138	507	486	− 4	498	592	+19		
M	210*	704	672	− 5	704	952	+35		
F	152	544	510	− 8	530	764	+44		
F	122	462	422	− 9	459	580	+26		
M	154	558	506	− 9	565	680	+20		
F	122	462	414	−10	459	522	+14		
M	122*	470	415	−12	480	604	+26		
	Average, 25 rats			− 2			+34		
							(Necrotic)		
M	174	610	612	± 0	616	786	+28	150	−76

* Represents complete occlusion of aorta

urea concentrations of the hypertensive rats were 40 3 to 50 0 mgm per cent, the usual values for nonhypertensive rats 20 days after unilateral nephrectomy

Proteinuria is not ordinarily present in coarctation of the aorta   Nor was it found in these hypertensive rats (average 1 3 mgm per day (females), as compared to the controls of 1 1 mgm per day)

DISCUSSION

Partial occlusion of either the aorta or one renal artery in rats is followed by cardiac hypertrophy only when there is living renal tissue beyond the occlusion   the left ventricle undergoes hypertrophy relative to the right, and other causes of cardiac hypertrophy are absent, under these conditions we feel justified in stating that such rats are hypertensive   Proteinuria does not occur and renal function is normal as judged by blood urea concentration, just as in coarctation of the aorta   The nutritional state of the animals is not impaired   On the other hand, when renal tissue is absent distal to the occlusion, hypertension does not occur even though blood must flow in the presence of the same degree of mechanical obstruction

Just as the pressure head beyond the stenosis of the aortic isthmus is lower than that proximal to the stenosis so Blalock and Levy (18) have shown that the pressure beyond a partial occlusion of the renal artery is lower (by 50 per cent) than the general arterial pressure   It is not known whether the important hypertensive factor in interference with the renal blood supply is local hypotension reduced pulse pressure, or reduced blood flow

The kidney beyond a partially occluded renal artery has often been spoken of as "ischemic" On the other hand its function is said to be normal   If Van Slyke *et al* (19) are correct in correlating renal function with blood flow, one or

TABLE IV

Experiment C   Partial occlusion of the left renal artery

Sex	Body weight	Weight of heart			Weight of kidneys				
		Pre-dicted	Ob-served	Devia tion of observed from pre-dicted	Pre-dicted	Left kidney		Right kidney	
						Ob-served	Devia tion of observed from pre-dicted	Ob-served	Devia tion of observed from pre-dicted
	grams	mgm	mgm	per cent	mgm	mgm	per cent	mgm	per cent
F	180	619	955	+54	590	135	−77	791	+34
F	154	549	756	+38	534	180	−67	952	+78
F	194	654	902	+38	619	426	−31	740	+20
F	122	462	591	+28	459	126	−73	650	+42
F	172	598	767	+27	573	174	−70	856	+50
F	174	603	730	+21	577	182	−69	770	+33
F	184	629	740	+18	598	184	−69	835	+40
F	254	801	940	+17	736	338	−54	1000	+36
F	196	659	742	+13	623	162	−74	720	+16
	Average, 9 rats		+28				−65		+39
F	228	739	795	+ 8	687	667	− 3	710	+ 3
F	214	704	760	+ 8	659	650	− 1	754	+14
F	190	644	680	+ 6	611	468	−23	675	+10
F	162	571	588	+ 3	551	514	− 7	668	+21
F	154	549	560	+ 2	534	560	+ 5	594	+11
F	170	593	584	− 2	569	570	± 0	596	+ 5
	Average, 6 rats		+ 4				− 6		+11

TABLE V

Experiment D   Partial occlusion of the aorta between the renal arteries

Sex	Body weight	Weight of heart			Weight of kidneys				
						Left kidney		Right kidney	
		Pre-dicted	Ob-served	Devia-tion of observed from pre-dicted	Pre-dicted	Ob-served	Devia-tion of observed from pre-dicted	Ob-served	Devia-tion of observed from pre-dicted
	grams	mgm.	mgm.	per cent	mgm.	mgm.	per cent	mgm.	per cent
M	150	548	748	+37	555	296	−47	774	+40
M	132	499	655	+31	507	128	−65	834	+65
M	122	470	606	+29	480	252	−48	608	+27
M	122	470	606	+29	480	396	−17	576	+20
M	132	499	640	+28	507	240	−53	698	+38
M	320*	962	1130	+17	948	514	−46	1240	+31
F	204	679	762	+12	639	196	−69	886	+39
	Average, 7 rats			+26			−49		+37
M	206	694	754	+ 9	694	652	− 6	696	± 0
M	194	663	725	+ 9	666	665	± 0	692	+ 4
M	202	684	738	+ 8	685	666	− 3	744	+ 8
M	180	626	673	+ 7	631	598	− 5	622	− 1
M	172	605	638	+ 7	611	682	+12	700	+15
M	170	600	634	+ 6	606	580	− 4	572	− 6
M	160	574	590	+ 3	580	500	−14	544	− 6
M	192	658	680	+ 3	661	634	− 4	680	+ 3
M	128	487	500	+ 2	496	437	−12	484	− 2
M	176	616	630	+ 2	621	602	− 3	615	− 1
M	180	626	641	+ 2	631	624	− 1	672	+ 7
M	160	574	570	± 0	580	548	− 6	574	− 1
M	314	949	950	± 0	935	890	− 5	970	+ 4
M	292	899	880	− 2	889	772	−13	768	−14
M	320	962	940	− 2	948	864	− 9	890	− 6
M	305	928	876	− 6	916	872	− 5	825	−10
	Average, 16 rats			+ 3			− 4		± 0

* Represents complete occlusion of aorta.

the other of the above statements is incorrect Actually, neither renal blood flow nor renal function by rigid tests has ever been studied adequately under these conditions, Mason, Evers, and Blalock (20) have failed to demonstrate ischemia using A–V oxygen differences In dogs with ureteral obstruction, renal blood flow is uniformly reduced but hypertension does not always occur (21), while in still another variety of hypertension (after subtotal nephrectomy) rats have normal renal blood flows (22) It seems probable that in the Goldblatt dog, renal flow is near the lower limit of normal as long as hypertension is present.

Perhaps only the kidney, of all the tissues, has the power to raise general blood pressure when its blood supply suffers interference. Hartmann Ørskov, and Rein (23) showed that, in spite of

pressor reflexes and adrenalin, renal blood flow remains constant although flow through the leg undergoes simultaneous wide fluctuations, they suggest that the kidneys may regulate the circulation in the same sense as does the carotid sinus It is not clear whether this supposed regulatory action is explicable wholly on some metabolic process specific to renal tissue, or whether the peculiarities of the arrangement (24) of the renal blood vessel are also involved The present experiments on rats do not show whether partial occlusion of the aorta proximal to the celiac axis can cause hypertension of greater degree than distal occlusion, nor has it been proved that hypertension does not follow narrowing of the splanchnic arteries

Hypertension may be produced by partial occlusion of the blood supply of a completely dener-

TABLE VI

Experiment E.   Partial occlusion of the aorta between the renal arteries, with right (proximal) nephrectomy

Sex	Body weight	Weight of heart			Weight of left kidney		
		Pre-dicted	Ob-served	Deviation of observed from pre-dicted	Pre-dicted	Ob-served	Deviation of observed from pre-dicted
	grams	mgm.	mgm	per cent	mgm.	mgm.	per cent
F	117*	448	695	+55	447	552	+23
M	178	621	764	+23	626	738	+18
M	114*	447	534	+20	458	494	+ 8
F	119*	454	537	+18	452	526	+16
	Average 4 rats			+29			+16
F	140	512	561	+10	502	615	+22
M	166	590	642	+ 9	596	759	+27
F	112	434	463	+ 8	435	546	+25
M	116	453	482	+ 6	463	614	+33
M	200	679	714	+ 5	680	813	+20
F	126	474	500	+ 5	469	556	+18
F	144	523	546	+ 4	511	612	+20
M	136	510	530	+ 4	518	702	+36
M	168	595	618	+ 4	601	702	+17
M	177	618	640	+ 3	623	882	+42
M	198	673	688	+ 2	675	908	+35
M	150	548	554	+ 1	555	770	+39
M	143	529	532	+ 1	536	690	+29
M	168	595	596	± 0	601	746	+24
M	202	684	682	± 0	685	850	+24
M	190	653	644	− 1	656	824	+26
M	158	569	558	− 2	575	763	+33
M	93	385	378	− 2	397	433	+ 9
M	190	653	634	− 3	656	815	+24
M	168	668	643	− 4	670	763	+14
M	102	412	395	− 4	423	494	+17
M	134	504	478	− 5	513	674	+31
M	136	510	482	− 5	518	694	+34
	Average 23 rats			+ 2			+26

* Represents complete occlusion of aorta

TABLE VII

Experiment F   Partial occlusion of the aorta above both kidneys

Sex	Body weight	Weight of heart			Weight of kidneys				
						Left kidney		Right kidney	
		Predicted	Observed	Deviation of observed from predicted	Predicted	Observed	Deviation of observed from predicted	Observed	Deviation of observed from predicted
	grams	mgm	mgm	per cent	mgm	mgm	per cent	mgm	per cent
M	130	493	680	+38	502	502	± 0	480	− 4
M	142	526	676	+28	534	568	+ 6	667	+25
M	112	442	546	+23	452	412	− 9	435	− 4
M	172	606	736	+21	611	568	− 7	544	−11
M	122	470	568	+21	480	475	− 1	510	+ 6
M	114	447	536	+20	458	414	−10	445	− 3
M	158	569	666	+17	575	604	+ 5	648	+13
M	178*	621	726	+17	626	540	−14	556	−11
M	154	558	640	+15	565	535	− 5	532	− 6
M	162	579	666	+15	586	568	− 3	570	− 3
M	128	487	562	+15	496	474	− 4	460	− 7
M	160	574	657	+14	580	562	− 3	546	− 6
	Average, 12 rats			+20			− 4		− 1
M	170	600	652	+ 9	606	660	+ 9	660	+ 9
M	178	621	670	+ 8	626	552	−12	624	± 0
M	140	521	548	+ 5	528	517	− 2	527	± 0
M	175	613	638	+ 3	618	588	− 5	604	− 2
M	224	738	718	− 3	737	670	− 9	693	− 6
M	100	406	390	− 4	417	340	−18	350	−16
M	144	532	480	−10	539	466	−14	474	−12
	Average, 7 rats			+ 1			− 7		− 4

* Represents complete occlusion of aorta

vated kidney transplanted into the neck (18, 25), all the afferent nerves being severed, some chemical substance produced in the kidney must be involved   If the hypertension of coarctation is also brought about, as we believe, by a substance derived from the kidney, then we must explain how it is that this material increases peripheral resistance in the upper part of the body but allows a normal or even decreased resistance in the lower part   This need not be a stumbling block, for the all important factor in determining blood flow to a given tissue is the metabolic requirements of the tissue itself   Prinzmetal and Wilson (4) and Pickering (5) have demonstrated that when conditions in the arm tissues are such as to evoke hyperemia, then hyperemia occurs as adequately in hypertensive individuals as it does in normal subjects   In fact, it may be calculated from their data that peripheral resistance in the reactive-hyperemic arm of either a hypertensive or a normal subject is only 7 to 12 per cent as great as

the resistance in the same subject under control conditions   Since tissue needs for blood may reduce local resistance in generalized hypertension by 90 per cent, it becomes understandable that the resistance in the lower part of the body in coarctation may easily be reduced only as much as the usual 30 to 50 per cent (as compared with the upper part of the body)

The results of Pickering (5) show a similarity between patients with essential or chronic renal hypertension and those with coarctation of the aorta, while Prinzmetal and Wilson (4) found a difference   The latter felt that the hypertension of coarctation was of vasomotor origin, in distinction to the alleged peripheral origin of other varieties of hypertension (including that of acute glomerulonephritis in which Pickering (26) affirms a vasomotor origin)   In view of such divergent opinions, and since local conditions (including the metabolites produced locally) act to maintain an adequate local blood flow as long as the cardiac output is normal and the arterial pressure not greatly lowered, it seems unwise to attempt to draw conclusions as to the site of action of the agent causing hypertension in man from data on blood flow in the arm

Pickering's (5) explanation of the increased peripheral resistance in the upper body in coarctation of the aorta was a supposed failure of growth of that vascular bed, particularly the arteriolovenous anastomoses, such an assumption lacks supporting evidence and is not necessary

Blumgart et al (11) found no difference in arteriolar pressure between the arms and legs in coarctation of the aorta   This means only that the fall of pressure gradient occurred proximal to the site of measurement, since the method was an indirect one, the observation is of doubtful significance

SUMMARY AND CONCLUSIONS

A consideration of hydrodynamics indicates that the arterial hypertension which is present in the upper part of the body in coarctation of the aorta may not be explained upon the purely mechanical grounds of obstruction to blood flow In this condition, there is an increased resistance in the smaller vessels (arterioles) which receive blood from the aorta proximal to the stenosis of its isthmus   The cause of this localized increased

resistance is the same as the cause of the generalized increased resistance in a Goldblatt dog (with partially occluded renal artery), that is, interference with blood supply to the kidneys

This conclusion is supported by the production of hypertension (cardiac hypertrophy) in rats by partial occlusion of the aorta proximal to one or both renal arteries With partial occlusion of the aorta between the renal arteries, hypertension occurs only when living renal tissue is present distal to the occlusion, after simultaneous distal nephrectomy, hypertension never occurs even though there exists the same degree of mechanical obstruction to blood flow offered by the stenosis and presence of a collateral bed

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THE EFFECTS ON THE CARDIOVASCULAR SYSTEM OF FLUIDS ADMINISTERED INTRAVENOUSLY IN MAN   II   THE DYNAMICS OF THE CIRCULATION [1]

By MARK D ALTSCHULE and D ROURKE GILLIGAN

(*From the Surgical and Medical Wards and the Medical Research Department of the Beth Israel Hospital and the Department of Medicine, Harvard Medical School, Boston*)

Earlier work from this laboratory (1) has shown that appreciable increases in blood volume occur in man following the injection intravenously of isotonic or hypertonic solutions of crystalloids in volumes of 500 to 1500 cc.   It has been known for many years from studies in polycythemia vera (2, 3, 4, 5, 6, 7) that an abnormally large volume of circulating blood in itself causes no significant deviation from the normal in cardiovascular function   Studies of the effects on the cardiovascular dynamics of a rapid increase in blood volume, such as occurs as a result of the administration of large amounts of fluids intravenously are, however, fragmentary   In a few experiments Cohnheim and Lichtheim (8), as long ago as 1877, noted a rise in both venous and arterial blood pressures in anesthetized dogs as a result of massive intravenous infusions of physiological saline solution   Subsequently other authors (9, 10, 11, 12, 13) confirmed these findings   Meek and Eyster (11), Gollwitzer-Meier (12) and Onozaki (14) reported striking increases in the cardiac output of anesthetized animals following the rapid injection intravenously of large volumes of fluid   Observations in man are limited chiefly to studies of the pulse rate, and venous and arterial blood pressures (15, 16, 17, 18, 19)   In the present work the effect of the intravenous administration of crystalloid solutions on the more important measurable cardiovascular functions in man have been studied

MATERIAL AND METHODS

Thirty five observations were made on 34 subjects aged 17 to 71 years   twenty-six were males and eight females. Many of the patients studied were surgical cases who had had appendectomies, herniorrhaphies, or pelvic repairs   in these cases intravenous fluids were administered 3 to 6 hours postoperatively   Several of the subjects

were young convalescent male patients from the medical wards   these cases were essentially normal and agreed to receive intravenous fluid for the purposes of study   One patient was studied while receiving hypertonic saline in the treatment of peripheral vascular disease   studies were made in another patient who suffered from angina pectoris and was receiving fluids intravenously as an attempted therapeutic measure (20)   There were no evidences of cardiac decompensation, hypertension or of marked dehydration in any of the patients on whom observations are reported.   The results obtained in the surgical patients and in the unoperated group were the same   all studies therefore are treated as one group

The minute volume output of the heart was measured by the method of Starr and Gamble (21)   Studies of the cardiac output before and during the intravenous injections were made with the patient in the postabsorptive state and the semirecumbent position. The respiratory minute volume, tidal air, and basal metabolic rate were estimated in those patients on whom measurements of the cardiac output were made. The velocity of blood flow was estimated from the arm to tongue circulation time, according to the method of Winternitz, Deutsch and Brull (22)   The venous pressure was measured by the direct method of Moritz and von Tabora (23)   The pulse was counted for thirty-second periods the respirations for one minute periods   Measurements of arterial blood pressure were made by the auscultatory method with a mercury manometer and a standard arm cuff   A small calibrated spirometer was utilized to measure vital capacity   The effect of intravenous injections on the plasma and blood volume were studied as described in a previous communication (1)   The values for blood volume changes reported here were obtained by calculation from the serum protein hematocrit and estimated control blood volumes before injection   and the protein and hematocrit values after injection (Method A of our previous paper (1))   Electrocardiographic tracings were taken with a Hindle string galvanometer   The above measurements were made at frequent intervals during and after the injection of fluids   in instances where the changes were not great only the changes from the control level to the level at the end of injection are tabulated.

In analyzing the results obtained in this study   changes of less than the following magnitudes were considered insignificant—venous pressure, 2 cm. water   pulse rate, 6 beats per minute   systolic and diastolic blood pressure, 4 mm. Hg   pulse pressure, 4 mm. Hg   velocity of blood flow, 10 per cent. By the method utilized, the volume of

fluid delivered intravenously was measurable to within approximately 50 cc.

In twenty-three studies a total of 500 to 1500 cc. of 5 per cent solution of glucose in physiological saline was administered, nine subjects received 1000 to 1500 cc of physiological saline solution, two received 1000 to 1500 cc of 5 per cent glucose in distilled water, one received 500 cc. of a 3 per cent solution of saline.

In most of the experiments venous pressures were measured, decholin was injected for measurements of pulmonary circulation time, and blood was drawn for hematocrit and protein measurements by means of a three-way stopcock connected with the needle inserted in the arm vein utilized for delivery of the fluid When measurements were made before the total volume of fluid had been injected, the flow of fluid was stopped completely for two to three minutes for duplicate measurements of venous pressure and a single velocity measurement, after which fluid injection was resumed In some experiments the fluid was delivered in one arm and the antecubital vein of the other arm was utilized for the above measurements Blood pressure readings during injection were obtained in those experiments where the vein of only one arm was punctured. In calculating the average rate of injection for a given volume of fluid, the total time elapsed between the beginning of injection and the end of injection of that volume, including in many instances one or two brief interruptions for venous pressure or velocity measurements or blood sampling, was utilized

RESULTS

Pulse rate

Significant changes in pulse rate occurred in 14 of 33 studies (Table I) In eleven patients rises of from 6 to 18 beats per minute were found, in two others decreases of 6 beats per minute were observed The largest increases occurred in subjects receiving a liter or more of fluid, patients receiving hypertonic solutions exhibited a greater tendency toward acceleration of pulse than those receiving isotonic solutions Measurements of pulse rate made one-half hour after the end of the infusion in patients in whom rises occurred during injection revealed a tendency of the pulse rate to remain somewhat increased for this period

Arterial blood pressure

Measurements of arterial blood pressure were made during thirty experiments (Table I)

Significant increases in systolic blood pressure occurred in fifteen patients, in two cases the systolic pressure decreased

TABLE I

Changes in cardiovascular dynamics following the intravenous administration of fluids

Case	Age	Fluid Injected			Increases in					
		Kindll	Amount	Rate	Pulse rate	Arterial blood pressure		Venous pressure	Velocity of blood flow	Blood volume increase*
						Systolic	Diastolic			
	years		cc	cc per min ute	beats per min ute	mm Hg	mm Hg	cm H₂O	per cent	cc
L C	39	A	500	6	−6			0 7		10
O G	64	A	550	55	6	−2	−12			200
J M	41	A	600	23	8	6	−4	1.3	48	490†
M B	44	A	600	24	10	2	−6	1.2	20	320
E S	48	A	600	24	4	−18	−12	2 9		560†
H F	20	A	600	27	−2	6	0	1 0	6	390
A F	35	A	600	40	−4	18	10	−0 1		910
D L	44	A	600	60	6	14	8	5 4	16	570†
A S	43	A	700	64	−2	0	0	10.2	−4	620
L P	32	A	1000	9	0	2	2	0 0	−3	320
S G	17	A	1000	11				−1 0		500
A A	28	A	1000	15	4	2	4	−1.8	0	400
W S	71	A	1000	17	0	2	2	2 4		780
H S	52	A	1000	32	4	−2	−8	0 9	10	770
G H	22	A	1000	36	10	−4	−2	2.5	12	330
I F	27	A	1000	36				2 9		600
I O	17	A	1000	38	8	20	4	6 4	4	810
J Mo	22	A	1000	40	16	8	−8	3 1	11	900
T A	33	A	1000	43	4			3 4		880
J S	25	A	1000	43	0	4	2	6 9	12	470
L S	30	A	1000	61	8			6 0		560
M A	28	A	1000	71	18	6	0	7 6	12	910
W St	71	A	1500	16	0	2	−4	1.2		1320
L M §	57	B	1000	28	0	0	6	2 4		−40
S J	46	B	1000	29	0	6	2	1 6		320
R S	58	B	1000	29	2	10	0	2 6		430†
T L	44	B	1000	32	4	4	−6	4.2	16	320
B G	42	B	1000	33	2	10	2	3 0		
R. C	37	B	1000	35	2	−2	−2	−0 1	17	60
B C	57	B	1000	37	6	2	0	4 1		830
A So	52	B	1000	67	2	4	−6	0 6	13	170
B S	18	B	1500	58	10	4	4	0.8	0	880
J Mac	45	C	1000	53	−6	0	−2	6 0		990
A R	25	C C	1500	61	−2	10	2	1.3	−17	1090
G Hy	25	D	500	21	−2	−2	−2	−1.2	26	480

* Changes in blood volume have been calculated from the estimated control blood volume, the hematocrit and plasma protein findings before injection, and the hematocrit and protein values immediately after the injection of fluids according to Method A of our previous paper (1)

† These figures represent plasma volume increases.

‡ This study was made two days after the preceding one in this case

§ Uremia

‖ A = 5 per cent glucose in 0 85 per cent saline.

B = 0 85 per cent saline

C = 5 per cent glucose

D = 3 per cent saline

Fifteen patients exhibited changes in diastolic blood pressure, six showing a rise and nine a fall

The pulse pressure increased significantly in seventeen instances, decreased in two, and was unchanged in the remaining eleven cases studied Measurements of blood pressure made one-half hour after the end of injection in those patients whose pulse pressure showed an increase during the period of injection, revealed a tendency of the pulse pressure to remain increased for this period

Venous pressure

The venous pressure was normal before injection of fluid in every subject. During the injection of fluid at a given rate the venous pressure increased as the amount of fluid introduced increased, so that the change after the introduction of 1000 cc. was on the average approximately twice as much as after the introduction of 500 cc. (Figure 2)

Analysis of changes at the end of injection of 500 to 600 cc. of fluid in thirty two experiments showed greater increases in venous pressure at faster rates of injection (Figure 1) In 17 of these studies the rate of injection was below 40 cc. per minute, the changes in venous pressure were not appreciable with three exceptions (Figure 1) On the other hand seven of the fifteen cases receiving fluids at rates of 40 cc. per minute or greater showed increases of venous pressure

FIG. 2. RELATIONSHIP BETWEEN INCREASE IN VENOUS PRESSURE AND AMOUNT OF FLUID INJECTED

Solid lines represent cases receiving 5 per cent glucose in physiological saline solution, broken lines represent cases receiving physiological saline solution or 5 per cent glucose solution in distilled water

FIG. 1. RELATIONSHIP BETWEEN INCREASE IN VENOUS PRESSURE AND RATE OF INJECTION OF FLUID INTRAVENOUSLY, THE VOLUME ADMINISTERED WAS 500 TO 600 cc.

Dots represent cases receiving 5 per cent glucose in physiological saline solution crosses represent cases receiving physiological saline solution or 5 per cent glucose solution in distilled water

of 2.3 to 7.7 cm. of water after 500 to 600 cc. were injected.

In several instances in which 1000 cc. of fluid were injected the venous pressure increased to values above 12 cm. of water during the administration of fluid, in one subject a venous pressure of 19.2 cm. of water was obtained at the end of injection of 700 cc. of fluid at a rate of 64 cc. per minute.

The rise in venous pressure resulting from the intravenous administration of fluid depended to a considerable degree upon the nature of the fluid injected. For a given volume and rate of injection the venous pressure showed a tendency to increase more after hypertonic solutions (5 per cent glucose in 0 85 per cent saline) than isotonic solutions (Table I, Figures 1 and 3)

After cessation of injection the venous pressure, if increased started immediately to return toward the level obtaining before injection even in the instances where the venous pressure increased considerably, the values
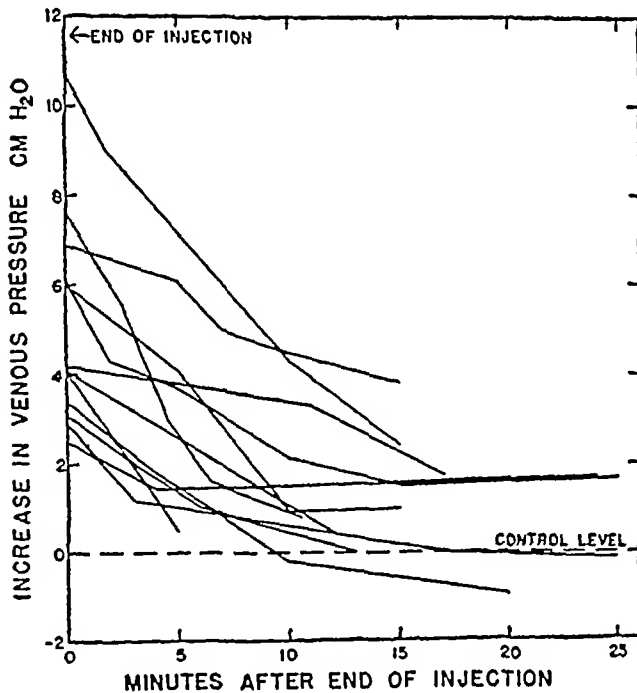
FIG 3 RATE OF FALL OF VENOUS PRESSURE TOWARD CONTROL LEVEL AFTER TERMINATION OF INJECTION OF FLUIDS INTRAVENOUSLY

Only those cases showing increases in venous pressure of 2 cm or more at the end of injection are represented

control figures in 10 to 25 minutes after the end of injection (Figure 3)

Velocity of blood flow

The arm to tongue circulation time was measured in 15 patients after 500 to 600 cc of fluid had been given intravenously Significant increases in velocity occurred in 10 instances, in 5 instances the increases were 20 per cent or more (Figure 4) The greatest increases in velocity were observed in cases in which the fluid was administered at rates of injection of 20 to 45 cc per minute (Figure 5)

A rough inverse relationship between rise in venous pressure and increase in velocity of blood flow through the lungs was found (Figure 4) Thus, four of nine patients who showed no appreciable increases in venous pressure after 500 to 600 cc of fluid had been injected showed increases in blood velocity of 20 per cent or more, on the other hand only one of six patients who did show appreciable increases in venous pressure exhibited an increase in velocity of blood flow of this magnitude (Figure 4)

Of fifteen patients whose velocity of blood flow was measured more than once during the course of injection, six showed a slower velocity of blood flow after receiving 1000 or 1500 cc of fluid than after receiving 500 or 1000 cc (Figure 5) In most of the other nine patients the maximum rate of increase in velocity of blood flow was observed during the injection of the first 500 cc of fluid, the curve of velocity increase against amount of fluid injected falling off as more fluid was injected (Figure 5)

Cardiac output

The effect of the intravenous injection of 400 to 1000 cc of fluid on the cardiac minute volume output was measured in six patients (Table II) The rate of injection of fluid in these cases varied from 11 to 36 cc per minute In three patients who showed no rise in venous pressure during the intravenous infusion the average increase in cardiac output per 100 cc of oxygen consumption was 10 per cent (Table II) The average increase in cardiac output in the three patients in whom rises in venous pressure occurred was approximately 40 per cent The increases in venous pressure noted in these subjects was small (Table II) The increases in cardiac output in these
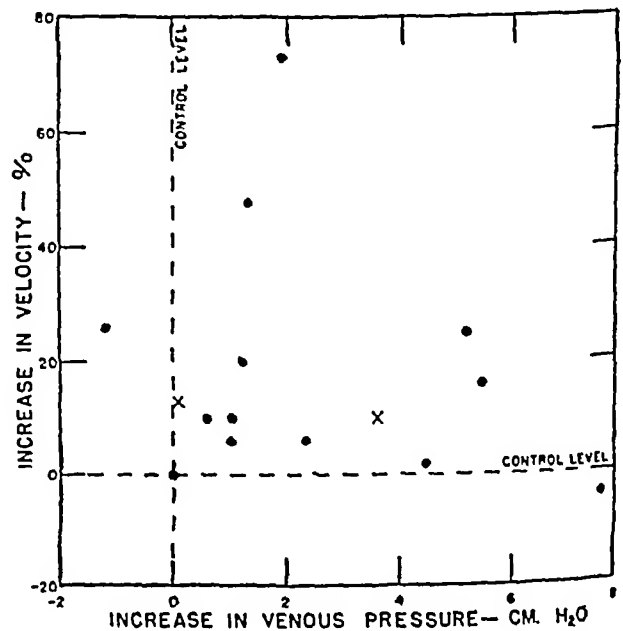
FIG 4 RELATIONSHIP BETWEEN CHANGES IN VELOCITY OF BLOOD FLOW AND IN VENOUS PRESSURE AFTER INJECTION OF 500 TO 600 CC. OF FLUID INTRAVENOUSLY
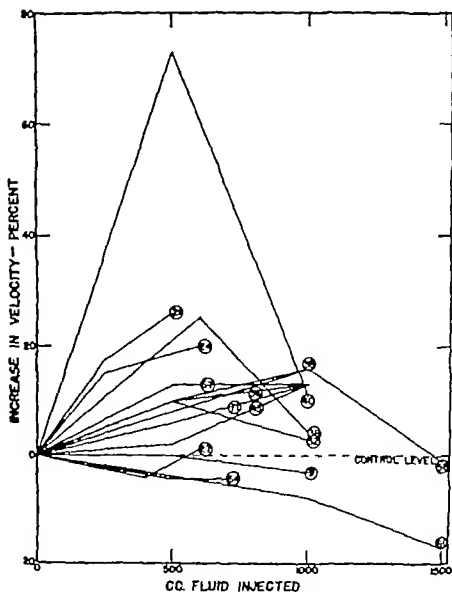
FIG 5 RELATIONSHIP BETWEEN INCREASE IN VELOCITY OF BLOOD FLOW AND VOLUME OF FLUID INJECTED IN SUBJECTS IN WHOM REPEATED MEASUREMENTS WERE MADE AT VARIOUS INTERVALS DURING INJECTION

The figures in circles indicate the rate of injection.

TABLE II

Changes in cardiac output and related aspects of the circulation during the injection of fluids intravenously

Case	Cardiac output per 100 cc oxygen consumed			Pulse rate		Arteriovenous oxygen difference		Rate of injection	Amount injected	Blood volume increase	Venous pressure increase	Velocity of blood flow increase
	Before injection	During injection	Increase	Before injection	During injection	Before injection	During injection					
	liters	liters	per cent	beats per minute	beats per minute	vol tenths per cent	vol tenths per cent	cc per minute	cc	cc	cm H2O	per cent
S G..	1.86	1.86	0	63	65	5.1	5.4	11	1000	500	−10	
G Hy	1.49	1.71	15	70	6	5.7	5.9	21	400	400†	−1	~6
A. A	1.5	1.75	16	61	53	6.6	5.7	15	1000	400	−1.5	0
J S.	1.74	2.18	25	66	63	5.7	4.6	20	800		+2.1	
H F	1.30	1.75	35	8	75		5.7	27	600	390	+10	6
G. H.	1.31	2.06	51	~0	~0	.5	4.9	36	1000	330	+2.5	1

* The composition of the fluid administered to case G Hy was 3 per cent saline all other cases of this Table received 5 per cent glucose in physiological saline solution

† The measurement of blood volume in this case showed an increase of 480 cc. after the injection of 500 cc. of fluid the value here presented is estimated

studies resulted entirely from increases in volume output per beat since the pulse rates remained unchanged

The velocity of blood flow was measured in three of the patients in whom cardiac output studies were made In one instance the increase in velocity was proportional to the rise in cardiac output in the other two cases in which the fluids were administered at more rapid rates the per cent increase in velocity of blood flow was markedly less than the per cent increase in cardiac output

The arteriovenous oxygen difference was strikingly diminished in the patients in whom the largest increases in cardiac output occurred lesser decreases were found in the other cases

Respiratory dynamics

The respiratory rate increased by more than two respirations per minute in only two of twenty-six studies

The other measurements of respiratory dynamics showed no consistent change The respiratory minute volume was measured in six cases In four cases it increased between 12 and 43 per cent, it was unchanged in the other two The tidal air was unchanged in three cases increased 40 per cent in one and decreased 20 per cent in two The vital capacity measured in four studies, showed no change

In no instance did the patient volunteer the information that he was short of breath, nor did direct questioning in several instances reveal the presence of dyspnea

The oxygen consumption studied in six cases during fluid injection was variable remaining unchanged in two cases decreasing 19 per cent in one and increasing an average of 25 per cent in three cases Two of the patients in whom increases occurred became obviously restless during the experiment

Electrocardiogram

Studies of the electrocardiogram by means of continuous tracings during the injection of fluid were made in ten patients who received 500 to 1000 cc. of fluid at rates of between 23 and 71 cc. per minute In five instances no changes occurred in the other five cases slight changes in the P or T wave, or in both were observed The
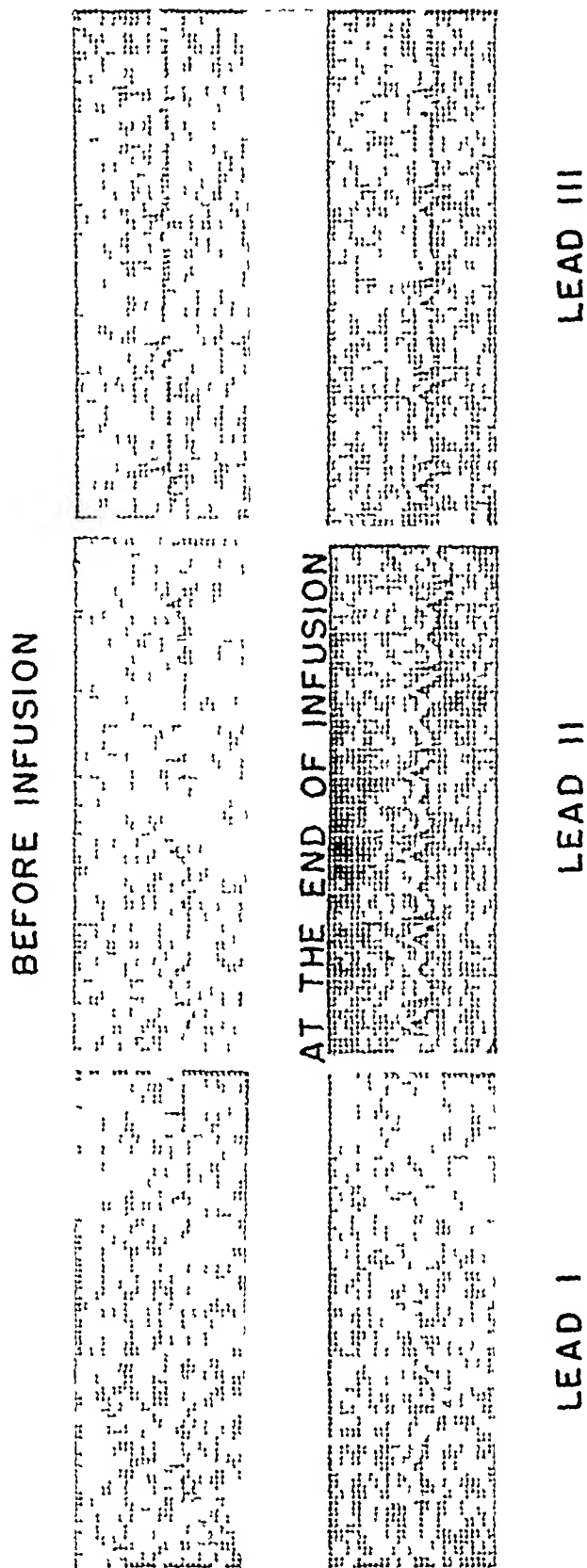
FIG 6 THE EFFECT OF THE INJECTION OF 1000 CC OF 5 PER CENT GLUCOSE IN PHYSIOLOGICAL SALINE ON THE ELECTROCARDIOGRAM OF CASE M A

size of the P wave in one or more leads increased in four instances, in one P^2 only was increased in two others P and P^3, and in the fourth P^1 and P^3 (Figure 6)   Changes in the T wave in one or more leads occurred in four instances, in three of these changes in P wave were noted also   In one case T^2 was increased, in a second T^1 and T^3 slightly increased, in a third T^1 decreased and in the fourth T^1 increased in size while T^* and T^2 diminished   Where changes occurred they were usually first observed when 400 to 700 cc. of fluid had been administered

Four of the five patients whose electrocardiograms showed changes received the injected fluids at rates ranging from 30 to 71 cc per minute   Two of the four patients whose electrocardiographic studies were negative received the fluid at a rate of more than 30 cc. per minute

In two cases where electrocardiographic tracings showed changes during injection, tracings were repeated two to three hours after the end of the injection, at this latter time the tracings were essentially like those before injection

DISCUSSION

It has been shown in a previous paper (1) and in Table I above that when fluids are injected intravenously under the conditions of this study a considerable increase in blood volume occurs and that the blood volume may not return to the control level until two hours after the termination of injection   The observations of the present study describe the various factors concerned in the adaptation of the normal cardiovascular system to the increases in blood volume brought about by fluid injections intravenously

When volumes of from 500 to 1500 cc. of fluid were injected at slow rates (from 6 to 20 cc. per minute) there occurred appreciable increases in blood volume, as great as 1300 cc in one case, but very little change in pulse rate arterial blood pressure, venous pressure and velocity of blood flow   On the other hand when fluid was administered at faster rates important changes in many of the cardiovascular measurements were found

When a liter or more of fluid was injected at rates greater than 20 cc. per minute the venous pressure generally became significantly increased. Several investigators have demonstrated in the

heart lung preparation and in the anesthetized intact animal that increase in venous pressure causes immediate increased output of the heart due to increased filling (9, 11, 24, 25 26, 27) In this investigation the cardiac output was measured in three instances in which the venous pressure was increased from 1 0 to 3 1 cm water by the injection of fluids intravenously   In these experiments the cardiac output increased from 25 to 54 per cent, the increase being accomplished through change in stroke volume. Presumably greater increases in minute volume output may have occurred in those cases showing greater increases in venous pressure.   The increased cardiac output found in this study closely accords with the findings in animals of Meek and Eyster (11) Gollwitzer-Meier (12) and Onozaki (14)   The results of Gollwitzer-Meier (12) and of Onozaki (14) in dogs and rabbits indicate that the cardiac output decreases to its control level within 15 to 30 minutes after cessation of injection

The changes in velocity of blood flow during injection of fluids were very variable   At moderate rates of injection the velocity of blood flow usually increased appreciably, with more rapid rates and larger volumes of injection however the increase in the velocity of blood flow was considerably less than expected on the basis of the observed changes in cardiac output   The fact that a longer time was consumed by the blood in traversing the pulmonary circuit than that expected with increased cardiac output indicates an increase in the total cross sectional diameter of the blood stream flowing through the lungs Stewart (28) has pointed out that the slower the pulmonary circulation time with a given cardiac output the greater the amount of blood in the lungs   That widely varying volumes of blood may be accommodated in the lungs by virtue of the elasticity of the pulmonary tissue has been suggested by Blumgart and Weiss (29)   Evidences of pulmonary engorgement sufficient to cause changes in vital capacity, or respiratory dynamics were not found in our subjects dyspnea did not occur

During the course of injection at moderate or more rapid rates the pulse pressure frequently increased progressively and the pulse became bounding   A progressive and diffuse flush was observed in almost all the cases receiving 1000 to

1500 cc of fluid These findings demonstrate peripheral vasodilatation following administration of fluids intravenously The aforementioned accumulation of fluid in the veins, as indicated by increase in venous pressure, and in the lungs, as indicated by the measurements of velocity of blood flow, when fluid is injected rapidly or in large volume must be regarded as owing to failure of the peripheral vascular bed to dilate sufficiently rapidly to accommodate the increased blood volume That this lag in peripheral accommodation occasions the increase in venous pressure observed under these conditions is evidenced by the observations that (1) blood volume may be increased more slowly to similar or greater levels without significant rises in venous pressure, and (2) when venous pressure does increase during rapid injections it returns to normal promptly after cessation of injection, although the blood volume remains increased Peripheral vasodilatation is, therefore, presumably the important factor in the final adaptation of the cardiovascular system to increased blood volume after intravenous infusions

In general, somewhat greater changes in the blood volume and in the cardiovasular dynamics were observed during injection of the solution containing 5 per cent glucose in physiological saline than when the isotonic solutions, plain physiological saline, or 5 per cent glucose in distilled water, were injected When solutions more hypertonic than 5 per cent glucose in physiological saline are injected intravenously for therapeutic purposes, the amounts given are usually small in volume The changes in blood volume and cardiovascular dynamics from such injections would, therefore, not be expected to be as great as many of the changes observed in this study Gibson and Evans demonstrated an increase in blood volume of approximately 200 cc during the first few minutes after the intravenous injection of 50 cc of 50 per cent glucose in saline (30)

The findings of elevated venous pressure and evidence of increased pulmonary blood volume in cases of this study are not interpreted as evidences of cardiac insufficiency The increases in cardiac output when fluids were injected rapidly were much greater than necessary to take up the increased fluid being delivered to the right heart,

increases in venous pressure and pulmonary blood volume, therefore, are not attributable to stasis resulting from myocardial insufficiency Further, a much decreased arteriovenous oxygen difference was found in subjects of this study with increases in venous pressure and in blood volume following intravenous infusions This is in contrast to the increased arteriovenous oxygen difference and elevated venous pressure resulting from decreased cardiac output in patients with congestive failure.

The finding of an increase in size of the P wave in the electrocardiogram (Figure 6) is interpreted as owing to increased electrical activity of the auricles, indicating increased work (31), probably a result of increased filling Changes in T wave, though found as frequently as those in P wave, were too variable in nature and degree to be amenable to interpretation

The results of this study and the interpretation placed on the findings describe the sequence of changes in cardiovascular dynamics after intravenous infusions of 500 to 1500 cc of crystalloid solutions in normal subjects As fluids are injected, there occurs a progressive increase in blood volume accompanied by a tendency toward increase in intravascular pressure Peripheral capillary vasodilatation intervenes, and when the rate of an injection is slow this mechanism accommodates the increased intravascular fluid volume to such an extent that other changes in cardiovascular status are not observable On the other hand, during the injection of fluids at faster rates, increases in the volume of the arterial, venous, and pulmonary portions of the circulation become manifest Within a short period following termination of these faster injections the venous pressure decreases in spite of persistent increase in the blood volume, indicating in these instances, also, final accommodation of the increased blood volume through capillary dilatation In our previous communication, it was shown that the blood volume returns to normal within approximately two hours after intravenous infusions as administered in this study (1) Increases in venous pressure result in increases in cardiac output which persist apparently for a period corresponding with the duration of the increase in venous pressure

Changes in cardiovascular function resulting from rapid intravenous infusions resemble in many ways the abnormalities in cardiovascular

dynamics owing to arteriovenous aneurysm   In both conditions, there is delivery of blood in increased volume and under increased pressure to the heart, with a consequent rise in cardiac output (32)   The occurrence of myocardial insufficiency resulting from continued increase in the work of the heart in patients with arteriovenous aneurysm is well known

The occurrence in elderly or cardiac patients of pulmonary edema and of angina pectoris as a result of the administration of fluids intravenously is not rare (33)   It is probable that the increased cardiac work resulting from intravenous infusions is the principal cause of the development of these complications in patients with a damaged myocardium   An additional factor favoring the development of pulmonary edema is the increase in the volume of blood in the lungs which occurs during the intravenous administration of large volumes of fluids   In patients with myocardial insufficiency and peripheral vasodilatation (34) associated with increased blood volume (35) rises in venous pressure and increases in the amount of blood in the lungs would presumably occur after smaller or slower intravenous infusions than those which produce these changes in normal subjects (11)   Richards *et al* (19) elaborating on Caughey's (17) earlier work observed a decrease in vital capacity, slowing of pulmonary circulation time, and onset of dyspnea during rapid intravenous infusions in some cardiac patients These authors also noted an abnormally great and prolonged rise in venous pressure following the injection of 1500 cc. of normal saline at a rate of approximately 50 cc per minute in patients with heart disease.

Intravenous infusions which continue for a period of several days impose conditions favoring the development of edema both peripheral and pulmonary, even though in such cases the fluid is given at very slow rates   The lowering of the plasma protein level due to plasma dilution, vasodilatation due to increased blood volume and the tendency toward increased venous pressure, by operating together over a period of days, may result in clinically perceptible edema (36)

The state of the cardiovascular system after the rapid injection of fluid intravenously is quite the opposite of that obtaining in shock   After intravenous injections, there are observed in creased blood volume, increased peripheral venous pressure, tendency toward increased systolic blood pressure, decreased arteriovenous oxygen difference flushing of the skin and bounding pulse, in shock, on the other hand, there occur decreased blood volume, collapse of peripheral veins, reduction of systolic blood pressure, increased arteriovenous oxygen difference, pallor and thready pulse.

Certain implications of the foregoing discussion are suggested in regard to therapeutics   When it is necessary to administer fluids intravenously to elderly, debilitated, or cardiac patients, the fluid injected should be isotonic, in small volume, and injected slowly, *i e*, at rates under 15 cc. per minute.   On the other hand, in the treatment of incipient shock when blood transfusion is not immediately available the crystalloid solution to be given intravenously should be hypertonic, in large volume, and injected rapidly, *i e*, at rates over 30 cc per minute.

SUMMARY AND CONCLUSIONS

1   The effects of the intravenous injection of isotonic and of slightly hypertonic crystalloid solutions on the venous pressure pulse rate, arterial pressure, cardiac output, velocity of blood flow, respiratory dynamics, electrocardiogram and blood volume of normal man have been studied

2   When 500 to 1500 cc. of physiological saline, 5 per cent glucose or 5 per cent glucose in physiological saline solutions, were injected at rates of less than 20 cc. per minute, very slight changes were observed in the cardiovascular functions studied, the blood volume was usually considerably increased

3   When these volumes of fluid were injected at more rapid rates considerable increases in venous pressure, cardiac output, velocity of blood flow, and in blood volume were usually observed, increases in pulse rate, pulse pressure, and in the P wave of the electrocardiogram were observed in some instances

4   The greater venous pressure increases occurred in subjects who received fluids in the larger volumes and at the more rapid rates   The venous pressure invariably returned to the control level within 10 to 25 minutes after the   l administration

5   Significant increases in cui

curred in patients in whom the intravenous injection of fluids resulted in rises in venous pressure

6 When fluids were injected in larger volume and at more rapid rates the increase in velocity of blood flow was considerably less than that expected from changes in the cardiac output In some instances the increase in velocity of blood flow was greater after the injection of 500 cc of fluid than after 1000 or 1500 cc These findings are interpreted as indicating an increase in pulmonary blood volume during injection Dyspnea did not occur, and changes in respiratory dynamics were not observed

7 The fact that rises in venous pressure did not persist, or even did not occur, in spite of increased blood volume, together with the observation of increasing diffuse flush of the skin, point to a progressive peripheral vasodilatation during the course of injection of fluids Additional evidence in this regard is the tendency toward increased pulse pressure observed in some subjects

8 The clinical implications of these findings have been discussed

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CHANGES IN BLOOD AND INTERSTITIAL FLUID RESULTING FROM SURGICAL OPERATION AND ETHER ANESTHESIA [1]

By JOHN D STEWART and G MARGARET ROURKE

(*From the Surgical Laboratories of the Harvard Medical School at the Massachusetts General Hospital Boston*)

Much attention has been given in the past to *the study of changes in the blood produced by* hemorrhage. Considerably less study has been devoted to changes in body fluid resulting from ether anesthesia though such findings as elevation of blood sugar, increase in hydrogen ion concentration of the blood, and reduction in plasma bicarbonate, have been well established The present work presents measurements of changes in quantity as well as concentration, of certain components of body fluid as found in 16 patients subjected to the traumatizing factors of major surgical operations and to ether anesthesia. The time chosen for obtaining data to compare with the preoperative normal values was just at the end of operation, while the patient was still anesthetized None of the patients studied showed evidence of more than mild shock or anoxemia during the study period

METHODS

On the morning of operation before administration of preanesthetic drugs the fasting patient was weighed Determinations were then made of plasma volume, body fluid 'available for solution of thiocyanate (1), hematocrit, plasma protein serum protein and serum albumin In addition, serum nonprotein nitrogen and serum sodium potassium, chloride, and bicarbonate were measured. These determinations were repeated at the end of operation while the patient was still anesthetized and before parenteral fluid had been given. In most instances blood loss during operation was measured using the method of Gatch and Little (2)

Plasma volume was determined by the technic developed by Gregersen and his coworkers (3) using the blue dye T-1824 the serum concentrations being measured spectrophotometrically Hematocrit readings were made by adding 4 cc. of blood to 1 cc of 1 1 per cent sodium oxalate and centrifuging in hematocrit tubes until no further change in the reading occurred precautions against loss of carbon dioxide being taken. Serum sodium was determined by the gravimetric method of Butler and Tuthill (5) serum potassium by Fiske s modified cobaltinitrite method in which potassium is reprecipitated as potassium acid tartrate (6) serum chloride by Wilson and Ball s method (7) carbon dioxide content of the serum according to Van Slyke and Sendroy (8) total nitrogen by macro-Kjeldahl (9) and serum nonprotein nitrogen of serum by micro-diges tion and nesslerization. Serum albumin was determined by the sodium sulfate method of Howe (10) From the total protein of the serum and the serum albumin col loid osmotic pressure was calculated, using the nomographic formula of Wells, Youmans and Miller (11) Oxygen capacity was determined on heparinized venous blood drawn without stasis and equilibrated with room air at room temperature (17)

Whole blood volume was calculated from plasma volume and hematocrit The validity of this calculation rests on the questionable assumption that the cell plasma ratio of venous blood is the same as that of blood in the capillary bed. It is probable that the capillary bed is relatively richer in plasma than is venous blood owing to axial streaming" of cells in the capillary flow (18) The computation is made, however in view of the pos sible comparative significance of figures obtained in the same individual in a short period. Total hemoglobin is computed from oxygen capacity and whole blood volume.

Body fluid available for solution of thiocyanate tentatively taken as extracellular fluid and comprising water of interstitial fluid, plasma, and red blood cells but not of cerebrospinal fluid, was measured by a modification Gregersen and Stewart (4) have made in the original technic of Crandall and Anderson (1) In this improved method the same sample of serum is used for spectrophotometric measurement of both the blue dye, T-1824 of the plasma volume method and the thiocyanate. Sodium thiocyanate is injected intravenously and a disappearance curve is constructed by determining the plasma level at ten minute intervals subsequently The point at which the curve flattens out, usually reached in 20 to 30 minutes is taken as the concentration in extracellular fluids after diffusion equilibrium has been established. In accordance with the suggestion of Lavietes Bourdillon, and Klinghoffer (13) correction is made for the slightly higher concentration of thiocyanate in serum than in transudate fluids The interstitial fluid values given in the table are computed by the following formula

Interstitial fluid =
        thiocyanate injected —
                (serum
            100/110 × serum

TABLE I

Percentile change given under initial preoperative value   Interstitial fluid volume calculated as fluid available for solution of thiocyanate minus whole blood volume

Case	Age and sex	Weight (kgm)	Operation	Duration of anesthesia (minutes)	Blood loss (cc.)	Blood loss / Blood volume (per cent)	Plasma volume (cc)	Interstitial fluid volume (cc)	Blood volume (cc)	Total plasma protein (grams)	Total hemoglobin (cc oxygen)	Hematocrit (per cent)	Oxygen capacity (volumes per cent)	Plasma protein (grams per cent)	Serum protein oncotic pressure (mm water)	Serum potassium minus (m eq per liter)	Serum sodium (m eq per liter)	Serum bicarbonate (volumes per cent)	Serum chloride (m eq per liter)	Serum nonprotein nitrogen (mgm per cent)
1	57 F	66.9	Gastric resection	160	256	7.4	2,350 / -33.8%	11,990 / +15.3%	3,419 / -32.0%	153 / -32.3%	410 / -35.9%	31.2 / +6.1%	12.0 / -5.8%	6.51 / -1.8%	280 / -3.6%	4.0 / -42.5%	140.2 / +3.8%	46.4 / -11.0%	118 / +3.8%	17.9 / 0%
2	27 M	73.2	Thoracoplasty	160	972	13.1	3,810 / -26.5%	7,230 / +45.5%	7,390 / -30.2%	311 / -25.4%	1,472 / -35.1%	48.5 / -5.5%	19.9 / -7.0%	8.17 / +1.6%		4.4 / -2.3%	139.7 / -1.6%	51.4 / -6.4%	100.3 / -1.8%	29.1 / -8.2%
3	49 F	61.4	Radical mastectomy	120			2,721 / -25.9%	7,290 / +29.2%	5,290 / -28.4%	225 / -32.5%	964 / -26.8%	48.6 / -3.7%	18.2 / +2.4%	8.25 / -8.3%		4.2 / -14.3%	141.2 / -1.5%		101.0 / -2.0%	22.1 / -14.9%
4	33 M	65.5	Thoracoplasty	70	461	9.9	2,802 / -25.7%	10,230 / +14.2%	4,618 / -27.5%	219 / -25.1%	806 / -34.0%	39.3 / -3.8%	17.5 / -9.1%	7.80 / -0.2%		4.4 / 0%	139.7 / 0%	54.8 / -8.8%	104.0 / -0.7%	27.7 / +2.1%
5	40 F	68.7	Vaginal hysterectomy	70	629	10.3	3,421 / -25.1%	6,360 / +70.6%	6,080 / -24.3%	235 / -25.9%	1,162 / -22.9%	43.8 / +11%	19.1 / +2.1%	6.85 / -0.8%	305 / -7.2%	4.6 / -13.1%	139.9 / -1.2%	57.1 / -18.6%	106.4 / -1.7%	20.4 / +16.2%
6	39 F	62.1	Total hysterectomy	140	440	8.9	2,857 / -24.6%	4,670 / +61.5%	4,890 / -24.1%	217 / -29.2%	842 / -26.9%	41.7 / 0%	17.2 / -3.4%	7.62 / -6.1%	360 / -8.9%	4.2 / -9.5%	137.6 / +2.2%	55.0 / -15.8%	104.4 / +2.6%	29.6 / +2.3%
7	62 F	61.8	Radical mastectomy	120			2,700 / -20.9%	5,880 / +37.1%												
8	45 M	68.4	Thoracoplasty	120	610	8.9	3,921 / -14.1%	9,010 / +65.9%	6,860 / -14.4%	273 / -13.3%	1,268 / -15.7%	42.6 / 0%	18.5 / -1.6%	6.96 / +1.1%	375 / -1.1%	4.3 / -9.3%	144.0 / -3.4%	55.0 / -10.0%	103.3 / 0%	26.0 / -5.0%
9	43 F	69.8	Colporraphy	125	579	11.7	2,773 / -13.5%	9,250 / +15.7%	4,920 / -13.4%	222 / -13.9%	892 / -16.5%	43.7 / 0%	18.1 / -3.3%	8.03 / -1.0%	380 / -4.5%	4.0 / -12.5%	137.5 / 0%	60.2 / -12.4%	106.0 / 0%	21.2 / -9.4%
10	26 F	60.6	Appendectomy uterine suspension	80	96	2.0	2,368 / -12.7%	7,450 / -12.3%	4,670 / -10.2%	196 / -12.7%	966 / -14.5%	49.3 / +2.8%	20.7 / -4.8%	8.29 / -0.3%			134.0 / +1.8%	62.6 / -12.6%	99.5 / 0%	24.9 / +1.2%
11	42 M	56.6	Herniorraphy	70	79	1.4	2,964 / -8.9%	5,250 / +43.6%	5,360 / -8.2%	212 / -8.0%	1,021 / -6.7%	44.7 / +11%	19.0 / +1.5%	7.17 / +0.8%	317 / -2.9%	4.0 / -15.9%	141.6 / -1.7%	66.2 / -9.2%	105.6 / 0%	24.2 / -13.2%
12	38, F	59.5	Radical mastectomy	130	546	10.5	3,103 / -5.8%	5,560 / +64.2%	5,194 / -3.3%	205 / -4.9%	883 / -10.8%	40.3 / +3.2%	17.0 / -7.6%	6.59 / +0.9%	274 / 0%	4.5 / -8.9%	134.5 / +1.1%	50.3 / -2.9%	104.0 / +2.4%	23.2 / -3.2%
13	35 F	60.5	Colporraphy laparotomy	100	297	5.4	3,110 / -3.2%	7,350 / +0.9%	5,440 / -4.8%	254 / -8.2%	887 / -2.0%	42.8 / -2.1%	16.3 / +3.0%	8.16 / -4.9%	390 / -6.4%	3.9 / 0%	136.2 / +2.7%	60.0 / -7.6%	102.5 / +3.9%	24.4 / +11.5%
14	47, M	79.6	Thoracoplasty	130	424	8.3	2,761 / -3.7%	7,800 / +22.6%	5,090 / -6.3%	225 / -4.4%	957 / -6.3%	44.8 / -2.9%	18.8 / 0%	8.14 / -1.9%	372 / 0%	4.8 / -12.5%	139.6 / +0.7%	53.5 / -8.7%	105.0 / -4.4%	21.3 / +28.6%
15	62, F	79.5	Radical mastectomy	120			3,082 / +1.7%	9,480 / +25.8%	4,715 / +1.2%	211 / +10%	584 / -12%	34.5 / 0%	12.4 / -2.4%	6.82 / -0.5%	324 / -0.3%	3.4 / -8.8%	138.1 / 0%	61.8 / -16.7%	104.0 / +0.9%	19.1 / +105%
16	17 F	52.2	Pelvic laparotomy	150	162	3.9	2,598 / +3.3%	7,980 / 0%	4,110 / +3.9%	194 / +5.1%	629 / +3.8%	36.8 / +11%	15.3 / 0%	7.17 / +1.7%	367 / -3.8%	3.3 / 0%	136.5 / -0.6%		102.6 / +1.0%	16.8 / +17%
Average percentile change							-14.9%	+31.2%	-14.8%	-15.1%	-16.7%	-0.17%	-2.4%	-1.2%	-3.5%	-10.7%	+0.35%	-10.8%	+0.36%	+7.3%
Mean percentile change							-13.8%	+27.5%	-13.4%	-13.3%	-15.7%	0%	-2.4%	-0.5%	-3.6%	-9.4%	0%	-10.0%	0%	+12%

It is probably of little practical importance that such a calculation ignores the fact that concentration of thiocyanate by volume is lower in red blood cells than in plasma owing to the lower water content of the cells, and that thiocyanate is excluded from cerebrospinal fluid.

DISCUSSION

The measurements obtained in this study are given in Table I In each instance the preoperative value is recorded and below it the percentile change from this value obtained from a measurement taken at the end of the period of operation and anesthesia. An outstanding fact described by these data is a considerable, and in several instances quite large, reduction of the volume of the blood without appreciable change in the physical or chemical character of the blood. In seven of the subjects, the reduction of total volume was more than 20 per cent, and the average for the series was 13 4 per cent. Hematocrit values were, however, found to be approximately normal and the structurally important components of blood plasma, namely protein sodium, and chloride, were found to be accurately sustained The stability of these concentration values makes it clear that the reduction of blood volume is not the result of a process of dehydration. In other words, blood volume is reduced *in toto* and not by withdrawal of water This reduction in most instances is, as may be seen in Table I, of much larger extent than is accounted for by the directly measured loss of blood caused by the operation Since this finding rests on the dye method of measurement it suggests that the quantity of actively circulating blood is reduced by a shunting of blood into some compartment of the vascular system which the dye does not rapidly enter The reduction of blood volume was in most instances in this series much larger than McAllister and Gregersen (12) found in dogs as a result of etherization for 1 to 2 hours This would be expected since to the effect of etherization is added a direct loss of blood and also the possibly contributing effect of tissue trauma.

The measurements of interstitial fluid volume obtained by the thiocyanate method describe a surprising and relatively large increase. The situation under study is one in which deficit in total body water must inevitably develop Water released by metabolic processes will fall far short of water expenditure by the lungs, skin, and kidneys This increase in interstitial fluid volume is of much greater extent than change in plasma volume and is in the opposite direction In view of the increase in volume of interstitial fluid indicated by the thiocyanate measurement one should expect either a reduction in the extracellular ions sodium and chloride, or else an increase in the extracellular concentration of the dominantly intracellular ion potassium Neither condition is present. Therefore the apparent increase in interstitial fluid is open to question, since there is no reason to suppose there has occurred an uneven distribution of ions between plasma and interstitial fluid beyond the relatively slight disproportion explicable on the Gibbs Donnan theory Nevertheless, the increase in interstitial fluid is so constant and large it deserves to be recorded It is possible that in ether anesthesia the accessibility of certain fluid compartments such as the intrathecal spaces, to the thiocyanate ion is increased

Other measurements recorded in Table I may be mentioned briefly The serum bicarbonate values show the quite well known moderate reduction The concentration of potassium was found to fall considerably We have no explanation to offer for this event, although in previous studies (14) increase of urinary potassium during operation and anesthesia has been found Lowering of serum potassium concentration has been reported in dogs following experimental hemorrhage without anesthesia (15) and in dogs and guinea pigs following experimental etherization (16) The approximately normal serum nonprotein nitrogen is not surprising According to the evidence of the measurements of oxygen capacity there is no appreciable disturbance of hemoglobin concentration

In considering these data one cannot but be struck by the extraordinary way in which the composition of the blood is stabilized, despite large volume changes due to such stresses on the homeostatic mechanisms as hemorrhage, etherization increased fluid loss through skin and lungs, and shifting changes in blood flow and cardiac output.

SUMMARY

In 16 patients undergoing major surgical operation under ether anesthesia   1   ?

hours, changes in plasma volume and interstitial fluid volume were studied   In 13 of these cases blood loss at operation was measured

Changes in hematocrit, oxygen capacity of venous blood, plasma protein, and colloid osmotic pressure of plasma protein were determined Alterations in serum potassium, sodium, chloride, bicarbonate, and nonprotein nitrogen were measured

From plasma volume, hematocrit, oxygen capacity and plasma protein were calculated blood volume, total circulating hemoglobin, and total circulating plasma protein before and after operation

CONCLUSIONS

1 The trauma of surgical operation and ether anesthesia lowers plasma volume as determined by Gregersen's dye method, and increases interstitial fluid volume, as measured by the thiocyanate technic

2 The reduction in plasma volume may be much greater than can be accounted for by hemorrhage

3 Concentrations of serum potassium and bicarbonate are significantly reduced

4 The tenacity with which concentrations of hemoglobin, and plasma protein, sodium, and chloride are held constant in the blood despite large volume changes is brought out

5 The fallacy of assuming a quantitative relationship between changes in concentration of hemoglobin or plasma protein and changes in plasma volume is brought out

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OBSERVATIONS ON THE BLOOD OF WORKMEN EXPOSED TO HIGH TEMPERATURES [1] [2]

By PAUL L. McLAIN AND EDWARD S MONTGOMERY

(*From the Department of Physiology and Pharmacology University of Pittsburgh Pittsburgh*)

The morbidity arising from exposure of workmen to excessively high environmental temperatures is an old problem, extended and accentuated by the development of modern industry. Economic and humanitarian motives have combined, in recent years, to stimulate investigation of the various clinical entities grouped under the general term " heat sickness " Of the many contributions on this problem, those of Hall and Wakefield (1), Bock and Dill (2), Talbott and Michelsen (3), Heilman and Montgomery (4), and Talbott (5) are particularly enlightening. The last author supplies an extensive bibliography. Both clinical and laboratory studies point to derangement of salt, sugar, and water metabolism as possible factors in the production of heat sickness

The present study amplifies the observations of Heilman and Montgomery (4) on the blood of steel workers. The blood findings of a control group were compared with those of patients exhibiting symptoms due to heat. Some technical modifications and additional observations necessitated an entirely new control series. The work occupied the summer months of 1936 and 1937

MATERIAL AND PROCEDURE

oup consisted of 43 workmen involved daily exposure to conch heat sickness is likely to de-:cts were aged 26 to 58 years, 41 years. The average height h extremes at 64 and 75 inches from 125 to 234 pounds, aver-pounds. The controls were, at unation, clinically free of any

symptoms attributable to heat. No restrictions as to diet or fluid intake were imposed, since average working conditions were desired. Blood samples were drawn, in each instance, before the subjects reported for work and at the end of an eight-hour shift. The post-work samples were used for comparison with blood findings in victims of heat sickness

The group of patients comprised 30 workmen admitted to the Emergency Hospital for relief of heat symptoms. These patients were classified clinically as victims of heat cramps (17 cases), heat exhaustion (6 cases), and heat retention or "stroke" (7 cases). The criteria for such classification are outlined by Heilman and Montgomery (4). Blood samples were obtained from the patients prior to treatment and studied in the same manner as those of controls. In addition, control samples were obtained from 16 of the patients when they were symptom free.

MEASUREMENTS AND METHODS

The following measurements were made, chiefly on samples of venous blood defibrinated by gentle stirring. Great care was exercised to avoid rough treatment during defibrination. In all cases, the blood after defibrination retained its venous hue

Red and white cell counts were made in duplicate or triplicate, the most consistent results being averaged.

Hemoglobin concentration was estimated in the majority of instances by the Haden Hausser apparatus (clinical model). In some cases, the Sahli instrument was employed. All values were expressed as grams of hemoglobin per 100 cc. of blood

Percentage volume of red cells was measured on undiluted blood by means of a power hematocrit, driven at high speed until the columns of sediment showed no further shrinkage

Specific gravity was measured by Gutl modification of the Barbour and Hamilt ing-drop method (7)

417

afternoon determinations were more widely distributed than the morning, the averages for the two series were almost identical In addition, 55 8 per cent of the controls showed changes less than 10 mgm per 100 cc over the eight-hour period, while 27 9 per cent showed increase and 16 3 per cent decrease beyond this limit The maximum changes were plus 45 and minus 44 mgm per 100 cc, and the averages were plus 12 and minus 10 mgm per 100 cc Eighty-six per cent of the subjects showed changes between plus and minus 20 mgm per 100 cc

Plasma carbon dioxide combining power Carbon dioxide capacity of plasma was studied in only 8 control subjects Although no abnormal values were obtained, the results suggested moderate depletion of alkali reserve over the work-period in 62 5 per cent of the subjects

Serum calcium Observations on serum calcium in 8 control subjects covered identical ranges and yielded identical averages in morning and afternoon series The maximum change noted was an increase of 0 2 mgm per 100 cc in one case The consistency of the results led to early abandonment of this determination

Summary of control series

The blood findings among the control subjects were within normal limits The outstanding changes over an eight-hour work-period were (*a*) increase in specific gravity of blood in about half the subjects, (*b*) increase in serum specific gravity in about three-fourths of the subjects, (*c*) decrease in blood sugar in nearly 60 per cent of the subjects A majority of the controls showed no significant change in red cell count, volume of red cells, color index, volume index, or serum chloride

II The blood in heat sickness

The blood findings in victims of heat sickness will be discussed according to the clinical diagnosis Table III summarizes the averages in this manner, and allows comparison with the afternoon control averages Table IV shows the direction of blood changes between the symptom-free and morbid state in those patients upon whom control studies were made Table V gives values from an example of each clinical type of heat disorder

TABLE III

*Comparison of averages in controls and victims of heat sickness **

Observation	Controls (P M)	σ	Cramps	σ	Exhaustion	σ	Retention	σ
					Patients			
Red cells (millions per cu. mm.)	5.232	0 44	5 000	0 00	5 700	1 01	5 000	0.22
Hemoglobin (grams per 100 cc)	14.3	1 13	15 5	1 05	14 6	0.57	14.8	0 46
Volume of red cells (per cent)	45 7	2 64	52.1	5 63	48 9	1.83	46 7	1.25
Color index	1 01	0 09	0 96	0 09	0 95	0 13	1 07	0 06
Volume index	0 99	0 08	0.99	0 07	0 99	0 12	1.04	0 06
Specific gravity of blood	1 055	0 003	1 063	0 005	1 060	0 004	1 057	0 001
Specific gravity of serum	1 026	0 003	1 031	0 007	1.030	0 005	1 027	0 002
White Cells (thousands per cu. mm)	7.80	1.51	11.27	2.10	6.85	2 18	8.96	1.59
Blood sugar (mgm per 100 cc)	78 6	11.2	97 5	24.8	63 6		100 0	
Serum chloride (mgm Cl per 100 cc)	381 6	16.6	325.3	55 7	350 6	35 5	406 7	22.6
Plasma CO₂ capacity (volumes per cent)	57.8		57.3		41 0		62.5	

* The symbol *sigma* refers to standard deviation

Heat cramps The highest individual values and the highest averages for red cell count, hemoglobin, hematocrit, white cell count, and specific gravity of blood and serum occurred among victims of heat cramps Averages for all these determinations were distinctly higher among cramp victims than among controls (Table III) In general, the individual values for these measurements were distributed in the upper half to two-thirds of the control range, with 17 to 41 per cent of values above this range and none below it Among 7 patients with cramps upon whom control tests were made, 4 to 6 showed, during symptoms, an increase in the blood constituents under discussion (Table IV) These results indicate the frequent occurrence of some degree of blood concentration among victims of heat cramps They indicate further that such concentration need not produce blood findings of abnormal character

Blood sugar during cramps ranged from 44 to 168 mgm per 100 cc, the extremes for all patients, regardless of type The average was considerably higher among cases with cramps than among controls, but 72 per cent of the determinations were in the control range All of the patients who served also as controls showed an increase of blood sugar during symptoms, but the changes were small

Of the chemical measurements, serum chloride

TABLE IV

Summary of blood changes in victims of heat sickness upon whom control observations were obtained

Diagnosis	Observation	Increased		Decreased		Unchanged	
		Cases	Per cent	Cases	Per cent	Cases	Per cent
Heat cramps	Red cell count	5	71.4	0	0.0	2	28.6
	Hemoglobin	5	71.4	1	14.3	1	14.3
	Hematocrit	6	83.7	0	0.0	1	14.3
	Specific gravity of blood	5	71.4	1	14.3	1	14.3
	Specific gravity of serum	4	66.7	0	0.0	2	33.3
	White cell count	5	71.4	0	0.0	2	28.6
	Blood sugar	4	100.0	0	0.0	0	0.0
	Serum chloride	0	0.0	6	85.7	1	14.3
	CO₂ capacity	1	25.0	2	50.0	1	25.0
Heat exhaustion	Red cell count	2	66.7	1	33.3	0	0.0
	Hemoglobin	1	33.3	0	0.0	2	66.7
	Hematocrit	1	33.3	0	0.0	2	66.7
	Specific gravity of blood	2	66.7	1	33.3	0	0.0
	Specific gravity of serum	1	33.3	2	66.7	0	0.0
	White cell count	0	0.0	1	33.3	2	66.7
	Blood sugar	0	0.0	3	100.0	0	0.0
	Serum chloride	0	0.0	0	0.0	3	100.0
	CO₂ capacity						
Heat retention	Red cell count	1	16.7	1	16.7	4	66.6
	Hemoglobin	5	83.3	1	16.7	0	0.0
	Hematocrit	1	16.7	0	0.0	5	83.3
	Specific gravity of blood	3	50.0	2	33.3	1	16.7
	Specific gravity of serum	1	16.7	5	83.3	0	0.0
	White cell count	3	50.0	1	16.7	2	33.3
	Blood sugar	1	25.0	0	0.0	3	75.0
	Serum chloride	2	33.3	1	16.7	3	50.0
	CO₂ capacity	0	0.0	0	0.0	3	100.0

proved most significant. Not only was the average distinctly lower among cramp patients than among controls, but 88 per cent of the patients showed values below the control average, and 35 per cent were below the lowest control figure obtained. Of 7 patients who were studied also as controls, 6 showed during symptoms, a decrease in serum chloride exceeding 18 mgm. per 100 cc., the maximum decrease being 157 mgm. It is apparent, therefore, that partial chloride depletion of the blood frequently accompanies heat cramps. However, there is no doubt that cramps of some severity may occur with a normal serum chloride. The serum chloride level need not reflect accurately the state of tissue chlorides.

Heat exhaustion. Among patients with heat exhaustion, the averages for red cell count, hematocrit, specific gravity of blood, and specific gravity of serum were significantly higher than the corresponding control averages. The range occupied by the individual values was similar to that already described for heat cramps; that is the results in general paralleled the upper two thirds of the control range. Values greatly exceeding the control range were exceptional in heat exhaustion. The most striking differences between controls and patients were noted in hematocrit and in spe-

cific gravity readings. For each of these determinations, 83 per cent of the patients exceeded the control average. These results indicate the frequent occurrence of dehydration among patients with heat exhaustion. The scarcity of extremely high values suggests that such dehydration was not as severe as that often accompanying cramps.

The blood sugar in heat exhaustion was below *the control average in 67 per cent of the patients* though all values were in the control range. The average among patients was 63 6 mgm. per 100 cc. while the control average was 78 6 mgm. per 100 cc. Furthermore, all cases upon whom control studies were made showed decrease of this blood constituent in excess of 13 mgm. per 100 cc.

TABLE V

Examples from various groups studied

Observation	Case 45 Normal		Case 53 Cramps		Case 50 Exhaustion		Case 54 Retention	
	A.M.	P.M	Control	Symptoms	Control	Symptoms	Control	Symptoms
Red cells (millions per cu. mm.)	5.0	5.2	5.0	5.2	5.5	5.1	4.9	5.0
Hemoglobin (grams per 100 cc.)	15.2	15.2	14.4	17.4	13.5	15.0	14.2	14.9
Volume of red cells (per cent)	49.5	49.0	50.0	66.0	47.0	48.5	47.5	48.0
Specific gravity of blood	1.060	1.063	1.058	1.073	1.053	1.059	1.056	1.055
Specific gravity of serum	1.028	1.032	1.031	1.042	1.028	1.029	1.027	1.030
White cells (thousands per cu. mm.)	10.2	8.9	9.8	18.2	5.0	5.2	7.7	9.2
Blood sugar (mgm. per 100 cc.)	134	102	89	96	63	44	52	100
Serum chloride (mgm. Cl per 100 cc.)	396	388	332	195	398	392	392	426
Plasma CO₂ capacity (volumes per cent)	76	52		62			62	53

Plasma carbon dioxide combining power was determined for 4 patients. In each case the result was below the lowest value encountered among controls. *The averages were 41 volumes per cent for victims suffering from exhaustion and 57 8 volumes per cent for controls.*

Heat retention. The blood findings in heat retention showed no important deviations from the pattern of the controls. Although the averages (Table III) for nearly all blood constituents examined were higher than the corresponding control figures, the difference in averages was significant from a statistical viewpoint, only in the case of serum chloride which was consistently high (above 400 mgm. per 100 cc.) in this type of patient. For all other measurements, the distribution of values failed to suggest real differences between controls and patients

SUMMARY

1 Red cell count, hemoglobin, per cent volume of red cells, color index, volume index, specific gravity of blood and serum, white cell count, blood sugar, serum chloride, plasma carbon dioxide combining power, and serum calcium were studied in (*a*) a series of symptom-free workmen exposed to high environmental temperatures, and (*b*) a series of patients presenting symptoms attributable to such exposure   In the control group, changes in these blood constituents over a work-period of eight hours were measured   Control values were compared with those obtained in victims of heat sickness

2 The results indicated (*a*) dehydration and (*b*) decrease of blood sugar over the work-period in a majority of controls

3 The most definite blood alterations encountered among victims of heat sickness were as follows (*a*) in heat cramps, tendency to blood concentration and decrease of serum chloride, the greatest deviations from the controls appearing in this group, (*b*) in heat exhaustion, tendency to blood concentration, decrease in blood sugar, and lowering of alkali reserve, (*c*) in heat retention, consistently high normal serum chloride

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THE NATURE OF THE LOWERED RESISTANCE TO INFECTION IN DIABETES MELLITUS [1]

By ALEXANDER MARBLE HAROLD J WHITE AND ALISON T FERNALD

(*From the George F Baker Clinic New England Deaconess Hospital, Boston*)

That| ~atients with diabetes have less resistance to infection than do normal individuals is a fact met with in the every-day experience of the clinician. Particularly common in diabetics are staphylococcic and streptococcic infections of the skin, *B coli* infections of the urinary tract, and tubercular infections of the lungs To be sure, since the introduction of insulin in 1922 and of protamine insulin in 1935, treatment has been so improved that excellent control of the diabetic condition is possible with almost all patients and the resistance to infection in well-controlled cases appears to approximate the normal However, prior to 1922 and today in patients whose diabetes is poorly controlled, the lessened ability to cope with infections cannot be denied

In the infections of the feet of elderly diabetics the factor of diminished blood supply resulting from arteriosclerosis is no doubt largely responsible for the slowly healing, non healing or gradually extending nature of such lesions However, allowing for this factor of poor circulation, the nature of the lowered resistance to infection still remains to a large extent unexplained It is the purpose of this paper to present data bearing upon this point

Various factors suggest themselves as the cause of the lowered resistance to infection in the diabetic. These may be summarized as follows

(1) Increased sugar content of blood and tissues

(2) Decreased activity of blood elements associated with resistance to infection (*a*) Subnormal activity of complement (*b*) Subnormal phagocytizing capacity of leukocytes, (*c*) Subnormal bacteriostatic and bactericidal action of whole blood

(3) Inadequate functioning of fixed tissue cells

(4) Lowered capacity of tissues to react to antigenic stimuli

(5) Lowered state of general cellular nutrition.

LITERATURE

One of the earliest ideas and one which is occasionally advanced even today is that the increased sugar content of the blood and tissues seen in diabetes provides a more favorable culture medium, particularly for staphylococci Such a view was held by Lassar (1) for example. Present-day opinion does not favor this explanation. Thus Handmann (2) found that *in vitro* blood containing 0.5 to 1 0 per cent sugar was no better culture medium for staphylococci than normal blood and that the addition of dextrose to blood within the limits found in diabetes did not decrease the bactericidal power of the blood or affect its opsonic index. Hirsch Kauffmann and Heimann Trosien (3) observed that streptococci pneumococci, and influenza bacilli grew better on blood agar plates made with blood from a patient in diabetic coma than on ordinary blood agar but there was no effect from hyperglycemic blood from non-coma cases, from the addition of dextrose and acetone *in vitro* or from cases of experimental hyperglycemia lipemia and acidosis. It is true that Kestermann and Knolle (4) reported that by the addition of sugar to normal serum in amounts which are found in the blood of diabetic patients the bactericidal effect of the serum toward colon bacilli could be appreciably reduced. However in similar experiments with staphylococci and streptococci such results were not obtained. It is possible that in the case of the colon bacilli, growth of the bacteria was favored by the enriched medium rather than by any lessened bactericidal power of the serum.

Although Pillsbury and Kulchar (5) working with induced staphylococcic skin infections in rabbits found that the frequent injection of hypertonic dextrose solutions caused an increase in the lesion they believed that the dehydration resulting from the injections of the hypertonic solutions rather than the dextrose itself was the responsible factor The same type and degree of increase of the lesion could be produced by injections of hypertonic salt solutions Polyuria and dehydration (accompanying prolonged and marked glycosuria) are regarded by Mosenthal (6) as responsible for the diminished resistance to infection seen in diabetes He as well as Bayne Jones (7) and Richardson (8) considers hyperglycemia *per se* as of little or no significant connection, although all will agree'

elevated blood sugar must be considered as a sign of uncontrolled diabetes and as the forerunner of acidosis

Richardson (9) with diabetic patients and Horster (10) with depancreatized dogs found that the amount and activity of the complement of the blood serum did not differ from that of normal blood   Bayer and Form (11) noted, however, that following pancreatectomy there was a decrease in hemolytic complement which could be temporarily restored to normal by the injection of insulin and dextrose

DaCosta and Beardsley (12), continuing the work of DaCosta (13), found, using Wright's technique, that with staphylococci, streptococci, and tubercle bacilli, the opsonic index of the blood of 50 diabetic patients was approximately one-third below normal   A high degree of glycosuria was found to imply a low grade of bacterial resistance as indicated by the opsonic index. Particularly with the tubercle bacillus, acidosis (which was present in 15 of the 50 cases) was associated with a low opsonic index.   It must be remembered that DaCosta's studies were carried out in 1908 before the time of insulin Sisto (14) using the same technique obtained in 1911 results similar to those of DaCosta and Beardsley except that in his experience the degree of glycosuria bore no relationship to the extent of the lowering of the opsonic index.   Horster (10) working with depancreatized dogs concluded that in these there was a functional disturbance of the leukocytes

Moen and Reimann (15) found that antibody production (typhoid agglutinins) was lower in patients with diabetes than in normal individuals, in proportion to the severity of the disease   Richardson (9) in a similar study of 42 diabetics and 39 non-diabetics came to the same conclusion   He, too, found that the poorer the control of the diabetes, the lower the agglutinin production   Wale and Madders (16), however, working with staphylococcal toxoid, found that diabetic blood had essentially the same amount of natural staphylococcal antitoxin and that following toxoid treatment, it developed virtually the same increase in antitoxin as normals did

Richardson (9) found that regardless of blood sugar level, diabetic blood had, in general, a lower bactericidal power than normal blood   The differences were not striking, however   In later work the same investigator (8) found that "underfed" rabbits with low liver glycogen showed lower typhoid agglutinin titers than did well-fed controls with higher liver glycogen   Hyperglycemia maintained by repeated doses of epinephrine had no significant influence on titers   He suggests that the lowered resistance of the diabetic may arise from a "disturbed cellular nutrition closely associated with the diminution of cellular glycogen reserve"

PRELIMINARY EXPERIMENTS

In an attempt to develop a suitable technique, various procedures were followed in the early part of the present study   Our purpose was to find a method by which we could demonstrate a significant difference in the behavior toward bacteria of diabetic blood, cells, or serum, as compared with that of normal individuals

At first, using the method of Ward and Enders (17) and freshly isolated strains of staphylococci and streptococci, the opsonizing power of the fresh, defibrinated blood of 10 diabetic patients was compared with that of normal controls   Using all possible combinations of washed normal and diabetic cells plus normal and diabetic sera, no significant tendency was found in favor of any combination   With the 2 strains of staphylococci used, the 2 types of sera and cells exhibited essentially the same marked phagocytic activity, and with 2 strains of streptococci (Lyons "M" strains), both kinds of blood were equally ineffective.

Tests designed to show differences in bactericidal power between normal and diabetic (defibrinated) blood using the method of Todd as modified by Ward (18) were equally inconclusive   We were unable to demonstrate even the slight differences between normal and diabetic blood reported by Richardson (9)

MATERIALS AND METHODS

The procedures eventually used for the bulk of the study represented attempts to show differences between blood from normal individuals and from diabetic patients by comparing the bacteriostatic, bactericidal, and phagocytizing action of the two types of blood upon Beta hemolytic streptococci   Since the preliminary experiments involving longer incubation had given essentially negative results, these later methods were designed chiefly to reveal any difference in bacteriostatic action initially (ie, within 2 to 6 hours) as demonstrated by a preliminary lag in bacterial growth   If such an early inhibition could be demonstrated with normal and not with diabetic blood, the results might be used to explain clinical events

Subjects   The non-diabetic controls were patients from the Outpatient Department of the Beth Israel Hospital, laboratory workers from the Harvard School of Public Health and the New England Deaconess Hospital, and 6 were patients in the Children's Hospital None had diabetes or, as far as could be determined, any other disease likely to be accompanied by a lowered resistance to infection   The diabetics were all patients in the New England Deaconess Hospital   The ages varied from 4 months to 65 years in the non-diabetic group and from 10 years to 70 years in the diabetic series

Bacterial strains   The following strains of Beta hemolytic streptococci were used   Wa, isolated from a human case of empyema, and classified as a member of Lancefield's Group A, Ward and Lyons' colony variant M, Ba and Sc kindly provided by Dr W S Tillett (see his paper (19) for description of these strains), NY5, a culture of the original Dochez scarlet fever strain   Capsules were easily demonstrable in cultures of Strains Wa, Ba, and Sc

Stock cultures   The strains were cultured in defibrinated horse blood, stored in the cold, and transferred monthly

Test cultures   Strains used in our tests were transferred at frequent intervals through buffered peptone broth containing 0 1 per cent dextrose   Young cultures, representing 3 to 5 hours' growth at 37° C. and contain-

mg an average concentration of 100 million bacteria per cc., were used to provide inocula in the various tests

Collection of blood Blood was withdrawn under sterile conditions from a vein in the antecubital space. The time of collection was usually in the late forenoon, 2 to 4 hours after the subject had had breakfast. No correlation between the length of time since food and the bactericidal effect of the blood was evident.

Working with horse blood no measurable difference was found in growth curves whether the blood was defibrinated or heparinized, and in view of the waste in defibrination heparinized blood was used. The subsequent addition of the bacterial culture to the blood was found to cause clotting unless an excess of heparin was present. A 0.5 per cent solution of heparin in 0 85 per cent sodium chloride solution autoclaved at 10 pounds pressure for 20 minutes was used. The addition of 0.5 cc. of this solution to 10 cc. of blood prevented clotting for 24 hours if the blood bacterial mixture was well shaken. Heparin rather than sodium oxalate, sodium citrate, or other anticoagulants was used since the reports of others (20 21 22, 23) indicated that in vitro the addition of heparin does not inhibit the growth of bacteria nor does it lower the phagocytic power of the blood.

Procedure for bacteriostatic and phagocytic tests Five cc. of fresh heparinized whole blood and 0 1 cc. of a 10^{-4} dilution in broth of a 3 to 5 hour broth culture of streptococci were placed in a sterile 25 cc. round bottom flask. A plate count on 0.5 cc. of a similar mixture was made immediately to determine the initial concentration of bacteria. Flasks containing test mixtures were then fastened in a horizontal position on a disc in the incubator (37 C.) Rotation at 10 r.p.m. insured continuous mixing of blood and bacteria during incubation. Pour plate counts using blood agar were made on 0.5 cc. samples taken after 2, 4 and 6 hours. Simultaneously with the tests for bacteriostatic action, mixtures were made using 0.5 cc. of the same blood and 0 1 cc. of undiluted culture to determine phagocytic capacity. The latter mixtures were rotated in the incubator at 37° C. for 30 minutes at which time smears were made to obtain phagocytic counts.

Procedure for bactericidal tests In addition to the tests mentioned under "Preliminary Experiments" further attempts to find a difference between normal and diabetic bloods in terms of bactericidal action were carried out as follows 2 cc. of heparinized whole blood plus 0 1 cc. of various dilutions of a 3 to 5 hour broth culture were mixed in flasks. In these tests the initial and final concentrations of bacteria were determined by diluting 1 0 cc. of test mixture quantitatively through 9.0 cc. blood broth dilution blanks (one drop of defibrinated normal horse blood in 9 cc. of broth) The test period was 24 hours All mixtures were continuously rotated at 40° C.

RESULTS

Bacteriostatic and phagocytic tests Having determined the most satisfactory type of medium for stock cultures and subcultures size and age of inoculum type of flask and hours of sampling by

an extensive series of preliminary tests with horse blood, 25 tests on 23 non-diabetic individuals and 27 tests on 27 diabetic patients were carried out to discover the difference, if any, in the inhibitory action and phagocytic power of the bloods upon Beta hemolytic streptococci The white blood cells were counted on all samples at the beginning of a test and on most of them at the end Streptococcus Strain Wa was used in all cases

Number	Subject			White blood cell count			30 minute phagocytosis (100 cells counted)			Logs of number of bacteria per cc.			
	Age	Sex	Blood sugar	0 hours	6 hours	Per cent left	Active phagocytic cells	Total cocci ingested	Index	0 hours	2 hours	4 hours	6 hours
	years		mgm. per cent				per cent						
1	50 ±	F	0 09	8,700	3,300	38	48	403	8.5	1.5	Sterile		
2	49	M.	0.09	11,000	4 900	45	54	663	7.9	2 4	1.0	1.0	Sterile
3	26	F	0.08	10 600			00	996	11.1	2.6	1.0	1.5	2.2
4	34	M.		6,800	1,000	35	44	312	7 1	3.0	1.7	1.5	3.0
5	41	F	0.13	12,700	4,500	35	73	612	8.5	2.4	1.0	1.0	2.5
6*	26	F		9,500	1,200	13	80	620	7.7	3.0	1.7	2.6	3.7
7	31	M.		8,200	2,100	-5	100	1,200	12.0	2.0	3.3	2.2	3.2
8	36	F	0.09	8,500	3,400	40	40	292	7.3	2.2	1.8	2.3	2.9
9	43	F	0.09	9,100	1,700	19	82	778	9.5	2.2	2.3	2.8	4.0
10	49	F.	0.10	8,700	2 400	28	23	108	3.4	2.3	2.4	4.4	5.3
11	31	M.		8,700	2 700	47	26	176	6.8	3.0	4.0	5.1	6.1
12	65	M.		6,900	2,000	29	44	255	6.1	2.5	3.5	4.6	5.7
13	37	F		13,500	5,900	44	60	828	13.5	3.2	4.3	5 4	6.5
14	53	F		8,000	6,600	83	55	782	13.1	3.1	4 4	5 6	6.8
15	46	F		11,000	5,500	50	76	1 132	14.9	3.1	4 4	5.5	6.8
16	29	F.		7,200	4,000	55	84	1,010	12.4	3.3	4	5.7	7 1
17	54	M.	0.10	7,600	1,800	21	8	48	6.0	2.3	3.6	5.0	6.3
18‡	31	M.	0.11	7 400			4	50	12.5	2.6	3.9	5 4	6.7
19	49	F	0.10	8,000	3,400	42	30	56	2.3	2.5	3.8	5.3	6.6
20	43	M.	0.11	9,000	3,200	36	12	45	4.0	1.5	2.1	4.6	6.4
21	51	M.	0.09	9 000	2,300	*5	18	212	12.0	1.5	3.4	4.9	6.4
22	11.5	F	0.09	8 500			16	243	16.0	2.0	3.3	5.3	7.2
23	11.0	F	0.08	7 600			8	62	8.0	2.0	3 6	5.2	7.2
24	7.5	F	0.07	11,200			22	204	14.0	1.5	3.4	5.2	7 1
25	8.0	F	0.03	6,700			6	96	16.0	.0	3.7	5.3	7 4

* In Tests 3 and 6 the same subject was used Test 3 was done 6 weeks after Test 6

† Phagocytic and bacteriostatic tests were carried out on separate mixtures simultaneously inoculated.

‡ In Tests 11 and 18 the same subject was used Test 18 was done 6 weeks after Test 11

The results obtained with the non diabetic individuals are shown in Table I. Tests 3, 4, 6, 7, 11, 18 were performed upon healthy young laboratory workers The subjects of Tests 3, 6, and 7 were in daily contact, and that of Test 4 in *weekly contact, with the streptococcus used* Tests 1, 2, 5 8 to 10, 12 to 17, 19 to 21, all inclusive were on patients from the C   t De partment of the Beth I▒   ▒▒ far as known, were suf▒▒

TABLE II

Phagocytizing and inhibiting action of heparinized blood of diabetic individuals upon streptococci (Strain Wa) †

Number	Date 1936	Patient Age	Sex	Diabetic history Duration (years)	Severity	Degree of control	Sugar of test blood (mgm. per cent)	White blood cell count 0 hours	6 hours	Per cent left	30 minute phagocytosis (100 cells counted) Active phagocytic cells (per cent)	Total cocci ingested	Index	Log of number of bacteria per cc 0 hours	2 hours	4 hours	6 hours	Remarks
1	December 1	68.5	F	1.4	Moderate	Poor	0.23	0,700	2,700	24	100	1116	114	2.3	1.0	Sterile	Sterile	Carcinoma of sigmoid refused operation
2	November 24	21.3	M.	0.3	Severe	Poor	0.31	12,200	8,500	70	100	1916	191	2.4	1.0	1.0	Sterile	Recurrent mild rhinitis and pharyngitis
3	December 8	46.9	M.	1.5	Moderate	Poor	0.21	9,800	3,300	34	33	225	7.0	2.3	1.8	1.3	2.0	No insulin until hospital admission 6 days before
4	December 8	16.8	F.	14.4	Severe	Poor	0.25	8,000	2,000	25	53	622	11.9	2.3	1.8	2.4	2.3	Admitted in diabetic coma 6 days before
5	December 11	61.0	F	3.0	Moderate	Fair	0.17	0,300			56	974	17.4	2.4	1.7	2.0	3.1	Admitted 3 months before with infection of foot and lymphangitis. Last operation 21 days before. No infection at time of test
6	December 2	34.9	F.	1.1	Moderate	Poor	0.22	6,000	1,000	28	16	200	12.9	2.6	1.3	2.7	3.3	Many W B C in urinary sediment since prostatectomy 22 days before
7	November 27	66.6	M.	22.7	Moderate	Fair	0.18	7,600	3,300	43	100	2,092	20.9	2.7	2.6	2.9	4.1	Severe diabetic neuritis 3 years before
8	November 24	38.3	M.	8.7	Severe	Fair	0.24	8,900	1,000	18	0	0	0	2.3	3.6	4.6	5.7	Admitted in diabetic coma 5 days before
9	November 24	9.9	F.	3.1	Severe	Poor	0.06	6,800	4,500	66	30	333	10.0	2.4	3.3	4.8	5.9	Diabetic coma 2 days before. Staphylococic urinary tract infection June 1936
10	November 24	14.7	M.	8.3	Severe	Good	0.30	7,900	4,800	61	90	1,221	13.6	2.2	3.3	4.8	6.1	Infection and gangrene of foot with lymphangitis on admission. Infection absent for 1 month prior to test
11	December 11	53.8	F	3.3	Mild	Fair	0.17	7,200			28	325	12.8	2.4	3.0	4.5	6.1	
12	November 19	13.3	F.	0.8	Severe	Poor	0.43	12,400	3,000	31	4	32	8.0	1.8	3.3	4.9	0.3	Admitted in diabetic coma preceding day
13	November 27	49.3	F	7.8	Moderate	Poor	0.28	0,200	3,500	38	90	1,674	18.6	2.6	3.5	4.9	0.3	Admission specimen of urine 3 days before, 7.3 per cent sugar
14	December 2	67.0	F	14.0	Moderate	Poor	0.29	11,700	3,200	26	18	174	9.7	2.6	3.0	5.2	0.3	Two weeks before, thigh amputation for infection of foot with lymphangitis. Chronic cystitis
15	December 2	64.8	F.	16.0	Moderate	Poor	0.40	10,600	4,300	42	18	222	12.4	2.0	3.8	5.1	0.3	Abscess right thigh. Chronic cystitis
16	December 1	62.7	F.	11.4	Moderate	Fair	0.29	7,000	2,400	31	0	0	0	2.1	3.6	5.0	0.4	Chronic B coli infection of urinary tract
17	November 27	70.2	F.	9.0	Severe	Fair	0.21	11,500	5,200	45	29	319	14.0	2.7	3.0	5.1	0.5	Mixed staphylococic and streptococic infection of right great toe. Acute pharyngitis during hospital admission
18	December 11	57.0	M.	0.3	Moderate	Good	0.21	8,700			30	416	13.8	2.5	3.7	5.1	0.5	Hemolytic streptococic infection of foot and lymphangitis 3½ mos. before with subsequent amputation of leg. Phlebitis left leg Nov 13-20. Infection absent 3 weeks prior to test
19	November 27	39.9	F.	5.4	Moderate	Fair	0.13	7,100	3,300	46	87	1,409	16.2	2.7	3.8	5.3	6.0	Acidosis 5 weeks before
20	November 24	14.5	F.	6.8	Severe	Poor	0.30	6,300	3,100	40	63	812	12.0	2.4	3.8	5.3	0.7	Diabetic coma 4 days before. Sept.-Oct. 1936 abscess right thigh
21	December 8	48.4	F.	10.1	Severe	Fair	0.14	8,100	1,000	23	12	83	0.8	2.3	3.1	5.1	0.7	Pulmonary tuberculosis
22	December 11	69.1	M.	0.4	Moderate	Fair	0.27	0,800			44	021	14.3	2.3	4.0	5.5	0.8	
23	December 11	57.7	M.	0.0	Moderate	Fair	0.16	8,300	3,600	49	0	0	0	2.3	3.9	5.6	0.8	Superficial infection of foot
24	December 1	42.3	M.	10.5	Moderate	Fair	0.23	7,300	3,400	30	10	83	5.5	2.3	3.9	5.3	0.8	
25	December 8	18.1	M.	6.5	Severe	Poor	0.22	11,500	3,000	45	18	130	7.2	2.3	3.5	5.3	0.8	Diabetic coma 2 days before
26	December 2	32.9	M.	8.9	Moderate	Poor	0.19	8,700			16	164	10.3	2.6	3.7	8.4	0.9	Carbuncle of nose infection of finger
27	December 18	19.5	M.	6.3	Severe	Poor	0.16	8,400			4	28	7.0	2.0	3.0	5.6	0.0	Diabetic coma July 1935 and Oct. 1936

* Urinary sugar said to date from 1882. Undoubted diabetes for at least 9 years
† Phagocytic and bacteriostatic tests were carried out on separate mixtures simultaneously inoculated

would alter the resistance of the individual to streptococcic infections The blood Wassermann test was negative in each instance. The children upon whom Tests 22 to 25 were carried out were in patients on the orthopoedic service at the Children's Hospital It is fair to state that, because of their physical handicap, these children although suffering from no disease other than structural abnormalities, might not possess average ability to cope with infections In each case, an attempt was made to ascertain whether the individuals had suffered streptococcic infections in the past A negative history was obtained in all instances except in the subject of Test 7 who had had a severe streptococcic lymphangitis in the left arm 7 months before, in that of Test 11 who had had streptococcic osteomyelitis 3 years before and that of Test 9 who complained of frequent attacks of pharyngitis

In all cases the number of white blood cells was definitely decreased at the end of the 6-hour period Of the 19 tests in which counts were made both at the start and finish, in 3 a final count of less than 20 per cent of the initial was obtained, in 5 from 20 to 29 in 3 from 30 to 39, in 5 from 40 to 49, in 2 from 50 to 59, and in 1 82 per cent. Tests 4 and 6 in Table I in which the greatest destruction of cells occurred showed respectively 44 and 80 per cent of active phagocytes, whereas the two tests showing the least cell destruction (14 and 16) had 56 and 84 per cent of active phagocytes The subject in Test 7 (mentioned in the preceding paragraph) showed 100 per cent phagocytosis and 25 per cent survival of white cells and inhibition of growth of the organism for 4 hours The bacteriostatic effect seen in Tests 3 and 6 possibly reflect the added resistance which this subject acquired through daily contact with the test cultures Only 2 of the normal bloods (1 and 2) sterilized themselves within the 6-hour period, and these showed 48 and 84 per cent active phagocytes, respectively

It is interesting to note that blood of the four children (Tests 22 23 24 25) showed negligible phagocytosis and a logarithmic increase in bacterial population in 6 hours.

The data as regards the diabetic patients are presented in Table II Twenty-one had initial and final white blood cell counts Of these the final count of one was 18 per cent, 5 were from

20 to 29, 5 from 30 to 39, 7 from 40 to 49, 2 from 60 to 69 and 1 was 70 per cent of the initial count Here as in the normal series there seems to be no correlation between the survival of leukocytes and the efficiency of phagocytosis In three tests (1, 2, 7) in which 100 per cent of the leukocytes were actively phagocytic, the percentage surviving at the end of 6 hours was 24, 70, and 42 respectively, and in 2 tests (8 and 16) where no cells were actively phagocytic, 18 and 34 per cent of the white blood cells survived Blood from 2 of the 3 patients showing 100 per cent phagocytosis brought about complete killing of the bacteria in 6 hours, but the third merely inhibited multiplication for 2 hours One case, 3, showed an inhibition of growth of the organism for the 6-hour period, but had only 32 per cent active phagocytes Two cases (4 and 6) showed inhibition of growth for two hours, with phagocytic counts of 52 and 16 The bloods showing the most rapid initial growth of the organism (12, 22, 24) had phagocytic counts of 4, 44, and 16 respectively

The 2 diabetic patients whose blood sterilized itself in the flasks were poorly controlled clinically Case 2 was a young man 21.3 years old, with diabetes of 9 3 years' duration, the blood used in the test had a sugar content of 0 31 per cent. Case 1 was an elderly woman 68 5 years old with diabetes of 1 4 years' duration, the sugar content of her blood used in the test was 0.23 per cent.

Cases 8 16, and 23 who showed no phagocytosis, all had 2 hour logarithmic increases in bacterial population of 1 4 which is not the greatest increase in the series nor was their 6-hour bacterial count as high as some (12, 22, 24, 25)

The phagocytic indices of the non-diabetics varied from 2.2 to 14 9 with an average of 9 7 The 2 bloods (1 and 2) which brought about complete killing of the bacteria had phagocytic indices of 8 5 and 7 9 In the diabetic group the indices varied from 0 to 20 9 with an average of 10.9, with the bactericidal numbers 1 and 2 showing indices of 11 4 and 19 1

The results obtained with the bacteriostatic and phagocytic tests are summarized in Table III As may be seen from Table III no significant difference was apparent between the behavior of diabetic and non-diabetic blood

Bactericidal power of blood

TABLE III

Summary of results of bacteriostatic and phagocytic tests

Type of subject	Total number of cases	Bacteriostatic tests						Phagocytic tests			
		Bactericidal*		Bacteriostatic†		Non-inhibitory		0 to 50 per cent active phagocytes		51 to 100 per cent active phagocytes	
		Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Non-diabetic	23	2	8 7	6	26 1	15	65.2	14	60 9	9	39 1
Diabetic	27	2	7 4	5	18 5	20	74 1	18	66 7	9	33.3

* Bactericidal sterility in 6 hours
† Bacteriostatic inhibitory to such a degree that the original population decreased at least during the first 2 hours and in most instances was either less or only slightly greater in 4 hours

of the work described above it was felt that the bacteriostatic and phagocytic tests did not indicate clearly any significant difference between the circulating blood of the diabetic and the non-diabetic individuals studied Further attempts to demonstrate such a difference in terms of bactericidal action were then carried out

Four bacterial strains were used Wa, Ba, Sc and NY5 (see description of organisms under "Materials and Methods") The blood of 12 non-diabetic and 6 diabetic individuals was available As can be seen readily from Table IV, the results obtained were essentially the same in the two groups Both had difficulty in exerting complete bactericidal action upon the resistant Wa and Ba strains and dealt about equally well with the less resistant strains Sc and NY5 One of the diabetic bloods (Number 3) killed all four organisms in 24 hours This patient is a diabetic of long standing, the first in Boston and perhaps in the United States to take insulin (on August 7, 1922) and her diabetes has never been under exceptional control It should be noted that these tests were carried out at 40° C With each test a horse blood control mixture was run on each organism at 40° C and in no case did it fail to grow

Using Strain Sc which all bloods tested killed at 40° C in a 10^{-4} dilution of a 4-hour broth culture, an attempt was made to determine whether normal blood would kill a larger inoculum of this organism than diabetic blood The results are shown in Table V

Of the 5 normals, Number 5 affected the culture in all dilutions but merely inhibited the un-

TABLE IV

Effect of human blood upon streptococci (Strains Wa, Ba, Sc, NY5) at 40° C *

Blood specimen number	3 hours incubation				24 hours incubation			
	Strain Wa	Strain Ba	Strain Sc	Strain NY5	Strain Wa	Strain Ba	Strain Sc	Strain NY5
A. NORMAL CONTROLS								
1	X	X	+	X	X	X	+	X
2	0	X	+	X	0	X	+	X
3	+	+	+	+	+	+	+	X
4	0	X	+	+	0	X	+	X
5	0	X	+	+	0	X	+	X
6	+	+	+	+	0	+	+	X
7	0	0	+	X	0	0	+	X
8	X	X	+	X	+	X	+	+
9	0	X	+	+	X	X	+	X
10	X	X	+	+	X	X	+	+
11	0	X	+	X	0	X	+	+
12	X	X	+	X	X	X	+	+
Totals	2+ 4X 6 0	2+ 9X 1 0	12+ 0X 0 0	6+ 6X 0 0	3+ 4X 5 0	2+ 9X 1 0	12+ 0X 0 0	4+ 8X 0 0
B. DIABETIC PATIENTS								
1	0	X	+	X	0	X	+	+
2	X	X	+	X	+	X	+	+
3	X	+	+	X	+	+	+	+
4	0	X	X	X	0	X	+	+
5	0	X	X	X	0	X	+	+
6	0	X	X	X	0	X	+	+
Totals	0+ 2X 4 0	1+ 5X 0 0	3+ 3X 0 0	0+ 6X 0 0	2+ 0X 4 0	1+ 5X 0 0	6+ 0X 0 0	6+ 0X 0 0

* + = Bactericidal effect, i e, no growth in subculture of test mixture
X = Bacteriostatic effect, i e, slight or no increase over initial concentration of bacteria
0 = No inhibitory effect, i e, logarithmic increase in concentration of bacteria

diluted inoculum The others except for Number 1, were not very effective In the diabetic series, 2 were bactericidal in all dilutions, while 4 were bacteriostatic for the undiluted inoculum and either bacteriostatic or bactericidal in the lower dilutions Hence here again no significant difference between the behavior of diabetic and non-diabetic blood was demonstrable

DISCUSSION

The results just outlined are of an essentially negative character in that they show no significant difference between the bactericidal, bacteriostatic, or phagocytic power of diabetic as compared with normal blood They demonstrate that if one car-

TABLE V

Effect of human blood upon streptococci (Strain Sc) at 40° C for 24 hours *

Blood speci men number	Dilutions of 3-hour culture of streptococci				
	Undiluted	10⁻¹	10⁻²	10⁻³	10⁻⁴
A. NORMAL CONTROLS					
1	0	+	+	+	+
2	0	0	0		
3	0	0	+		
4	0	+	+		
5	×	+	+		
B DIABETIC PATIENTS					
1		0	+	+	+
2	0	0	0		
3	+	+	+		
4	0	0	0		
5	0	+	+		
6	×	+	+		
7	0	×	+		
8	×	×	+		
9	×	×		'	
10	×	×	+		
11	+	+	+		

* + = Bactericidal effect ₁ e no growth in subculture of test mixture.
× = Bacteriostatic effect ₁ e., slight or no increase over initial concentration of bacteria
0 = No inhibitory effect ₁ e. logarithmic increase in concentration of bacteria

ries out such tests using a few selected strains of streptococci, approximately the same variation of bactericidal or phagocytic power will be found among a group of diabetic patients, regardless of duration, severity, or state of control of the diabetes, as among a group of normal individuals se lected at random This variation is without doubt partly dependent upon former chance contacts chiefly during infections, between the individuals concerned diabetic or non-diabetic, and the specific (or to a less extent, related) bacterial strains used Among other factors aside from the pos sible influence of diabetes itself is the variable capacity of individuals to respond to antigenic contacts Our findings suggest that diabetic pa tients who successfully combat past infections thereby develop specific immunity to roughly the same extent as do non diabetic controls It is true that Moen and Reimann (15) and Richard-son (8), in work already referred to, found the development of typhoid agglutinins poorer in dia betic than in normal individuals Corresponding

studies using streptococci or their products cannot be carried out so that one must be content with the type of data presented in the present paper Furthermore, we believe that the development of agglutinins is not as significant from the point of view of actual protection as the type of immunity demonstrable by the methods employed in the present study

It must be emphasized that our results were ob tained by the use of a few selected bacterial strains Different findings might possibly be se cured using other techniques and other organ isms, but the possibilities in this regard are many, and in view of the frankly negative character of the results to date, we have not thought it worth while to pursue the question along this line.

Wherein, then, does the lowered resistance to infection of the uncontrolled diabetic lie? We have already conceded that the well-controlled pa tient may exhibit an essentially normal defense. It seems likely that the commonly occurring mal nutrition, dehydration, and acidosis of the poorly controlled diabetic may contribute to poor resist ance in a manner which is not reflected in the type of study here reported Perhaps also one should consider more specifically the functional integrity of the fixed tissue cells, i e, the mononuclear cells of the reticulo-endothelial system Their impor tant role in bodily defense no one will deny One is intrigued by the possibility that, in the uncon trolled diabetic, the hypercholesterinemia which is present not infrequently may be associated with a 'blockade' of the reticulo-endothelial system with consequent lowering of its efficiency Upon this point data are difficult to acquire and our own experiments are not relevant.

CONCLUSIONS

Fresh defibrinated blood and heparinized whole blood of diabetic patients were found to possess essentially the same phagocytic, bacteriostatic, and bactericidal power against selected strains of streptococci as blood from normal controls Re sults in individual cases could not be correlated with the duration severity, or state of control of the diabetes
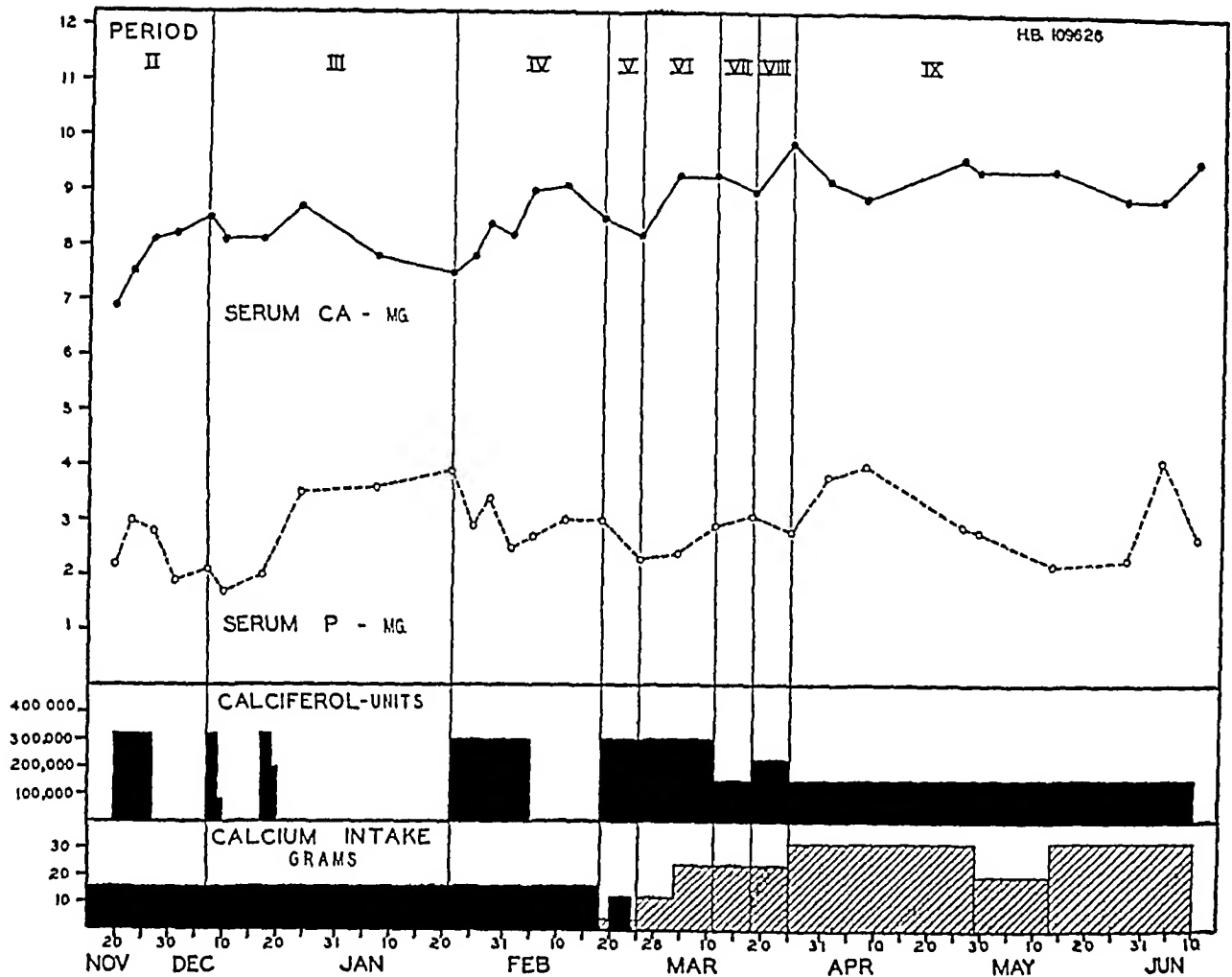
FIG 2 CASE I, H B POSTOPERATIVE PARATHYROID TETANY

Calciferol 1 mgm equivalent to 40,000 international vitamin D units Calcium salts Solid blocking = calcium chloride, shaded blocking = calcium lactate

only (December 13, 1935) was the serum calcium normal, but within a few days it had dropped again For a two-week period the patient was given a diet containing daily 2 0 grams of calcium and 0 6 gram of phosphorus (Diet A) There was a temporary rise in the serum calcium to 7 5 mgm per cent and a concomitant fall in the serum phosphorus to 4 8 but these promptly returned to their former levels at the end of a week Following this, a high acid-ash diet (Diet B) was tried for a period of three months As a result, there was a transient but small rise in the serum calcium and a fall in the serum inorganic phosphorus

On March 3, 1936, two parathyroid glands of pin-head size were removed from a still-born baby (3½ hours) and injected into the region of the left deltoid through a trocar with about 3 cc of Ringer's solution On June 17, 1936, a small parathyroid gland removed at operation was transplanted over the right femoral vein through a small incision in the skin No appreciable effect upon the clinical course or serum calcium was noted Five months af-

ter the second transplantation the serum inorganic phosphorus had fallen to 2 2 mgm per cent Since all the preceding and subsequent determinations were higher (1 c, when the patient was not taking calciferol), there is reason to question the accuracy of this determination

The average values for the entire period were serum calcium 6 4 mgm per cent, serum inorganic phosphorus 4 9 mgm per cent

Period II (17 days) For one week, daily doses of 320,000 vitamin D units of calciferol were administered The daily intake of calcium (CaCl, 16 grams) and vi-osterol (2 0 cc) was the same as in Period I Within three days the serum calcium had reached 8.2 mgm per cent, the highest it had ever been, except on the one occasion mentioned above The serum phosphorus at first rose slightly and then fell, but all determinations remained within normal limits during this period Calciferol was discontinued at the end of a week but during the next eleven days the serum calcium continued to rise, the phosphorus to fall

Period III (45 days) The viosterol and calcium in take remaining the same as in Period II, calciferol was given for three days (total 720 000 units) Eight days later a similar dose was given. There was no change in the serum calcium after the first block of calciferol, but following the second block there was a definite rise to a level of 8.7 mgm. per cent. Following the last dose of calciferol there was a gradual fall in the serum calcium, but even at the end of thirty three days it had not reached the average level of Period I. The serum inorganic phosphorus fell slightly after the first block of calciferol and rose after the second, although not to an abnormal level

Period IV (28 days) The calcium intake remaining the same as in Periods I II and III a daily dose of 300 000 vitamin D units was given for fourteen days twice as long as in either Period II or III There was a correspondingly greater rise in the serum calcium, reaching a maximum of 9 0 mgm. per cent. The serum inorganic phosphorus fell abruptly to 2.5 mgm. per cent. During the following week the serum calcium remained about the same and then fell, reaching a level of 8.5 mgm. per cent two weeks after the last dose of calciferol The serum inorganic phosphorus rose slightly (to 3 0 mgm. per cent)

Period V (7 days) Keeping the calciferol intake at the same level as in Period IV (300 000 units daily) the calcium intake was reduced to 4 grams of the lactate daily Within two days the patient developed acute tetany Unfortunately she was unable to come into the clinic, but increased her calcium intake to 12 grams of the chloride, with diminution in her tetany Despite the fact that similar doses of calciferol had previously resulted in a rise in the serum calcium, at the end of one week there was no rise but in fact, a slight fall. Had a blood calcium been determined on the third day of the period when the patient was in active tetany it would undoubtedly have been considerably lower This appears to demonstrate the dependence of calciferol activity on the calcium intake.

Period VI (14 days) With the same calciferol intake as in Period V, and 12 grams of calcium lactate daily there was a prompt rise in the serum calcium to 9.3 mgm. per cent. Doubling the calcium intake resulted in no further rise. At any given dose of calciferol, there appears to be an optimal calcium intake above which additions will cause no further rise in the serum calcium.

Periods VII and VIII (7 days each) The calcium intake was kept the same as in Period VI The calciferol dosage, however was reduced to one-half (150 000 units daily) At the end of one week there was a slight fall in the serum calcium. By increasing the dose to 225 000 units per day it was possible to raise the serum calcium again to 9 9 mgm per cent This response was of the same order of magnitude as that which resulted from a daily dose of 300 000 vitamin D units There appears therefore, to be an optimal dose of calciferol at any given intake of calcium, which will raise the serum calcium level.

Period IX (77 days) It was possible to maintain the serum calcium at approximately 9.0 mgm. per cent and to keep the patient free from tetany for a period of eleven weeks on 150 000 units of calciferol and 20 to 32 grams of calcium lactate daily

During the periods in which calciferol was administered, a total of 202 days, manifest tetany was absent at all times, except in Period V when the calcium intake was greatly reduced The only evidence of latent tetany which could be occasionally demonstrated was a slightly positive Trousseau s sign after application of a tight tourniquet for a period of more than one minute, a finding of questionable significance. The patient felt perfectly well during this period and exhibited no signs or symptoms of vitamin D intoxication The nervousness tremor and exophthalmos which were undoubtedly related to a state of hyperthyroidism, remained unchanged. There was slight persistent tachycardia, but no loss of weight. In December 1936 a small nodule appeared in the region of the left lobe of the thyroid. This increased somewhat in size during the ensuing three months and then remained stationary No iodine was administered to avoid complicating these studies A fluoroscopic examination of the chest in November 1936 failed to demonstrate a substernal thyroid.

Except for three months during Period I, the patient ate a well balanced diet of her own choosing Since no effort had been made to control its calcium or phosphorus content, the diet for a sample period of one week during June 1937 was recorded and analyzed. The average daily calcium intake (exclusive of added salts) was 0.566 gram the average daily phosphorus was 0.855 gram.

Case 2

J La P., a 39-year old Italian para iii, gravida iv, was delivered on August 9, 1936 of a normal full term male child, weighing 2710 grams after a labor of 6½ hours. The last week of pregnancy had been complicated by a severe pyelitis On the seventh day postpartum, the symptoms of pyelitis having already subsided the patient developed excruciating pain, numbness and spasm of the right hand and wrist. Examination of the blood revealed hypocalcemia and hyperphosphatemia, and Chvostek s and Trousseau s signs were positive confirming the diagnosis of idiopathic parathyroprivic tetany

The patient s first two pregnancies (1927 and 1929) had been uneventful. The last, in 1933 had been followed by a similar attack of tetany lasting six months and incorrectly diagnosed as arthritis as subsequent events proved. Since then she had had monthly attacks of tetany characterized by tingling and twitching of the thenar eminences, coinciding with the menstrual periods. The attacks were always mild involved the right hand more than the left, and usually appeared two or three days before, and subsided immediately after the onset of menstrual flow There was nothing else of note in the past or family history

Physical examination (three weeks after delivery August 29 1936) —The patient was a well developed obese Italian woman who did not appear ill. The vital signs were normal Scalp hair was abundant and of norm l

texture. The skin was dark, warm, and moist The nails were all present and showed no trophic changes Examination of the eyes revealed no evidence of cataract, the pupils and fundi were normal The teeth were in good repair and exhibited no unusual pitting or ridging The thyroid gland was of normal size and consistency and there were no eye signs, tremor, tachycardia, or excessive sweating to suggest thyroid disease The heart and lungs were normal, the blood pressure 120/80 Abdominal examination revealed right costovertebral angle tenderness but the kidneys were not palpable There was a mild cervicitis and a relaxed pelvic floor Slight pitting edema was demonstrable over the left ankle. Chvostek, Trousseau, and peroneal signs were markedly positive All the reflexes were hypoactive and equal, the Babinski physiological

Laboratory data—Blood Red blood cells 4,300,000, hemoglobin 11 4 grams (Sahli), white blood cells 13,600 On subsequent examination the red blood count, white blood count, and hemoglobin fell to 3,560,000, 7,900, and 10 4 respectively Urine yellow, cloudy, albumin 2 +,

sugar absent Microscopic loaded with clumped white blood cells, no red blood cells or casts Urine culture *B coli communior* Wassermann and Kahn blood tests negative Abdominal x-ray on September 2, 1936 kidney shadows of normal size and shape, no evidence of calcification in the kidneys or along the course of the ureters Phenolsulphonephthalein excretion test on June 5, 1937 (1 cc. intravenously) 15 minutes 25 per cent, 30 minutes 20 per cent, 1 hour 20 per cent, 2 hours 15 per cent Total excretion 80 per cent. Urea clearance 52.2 cc. per minute or 69 6 per cent. Creatinine clearance 92 8 cc. per minute

Several determinations of the serum total proteins, albumin globulin ratios, carbon dioxide combining power, chlorides, and nonprotein nitrogen were within normal limits

Course (Figures 3 and 4)—The patient ran a hectic temperature with marked pyuria for one week. These subsided under a régime of forced fluids, sodium acid phosphate, and hexamethyleneamine Subsequently, in the Outpatient Department, the latter was alternated with
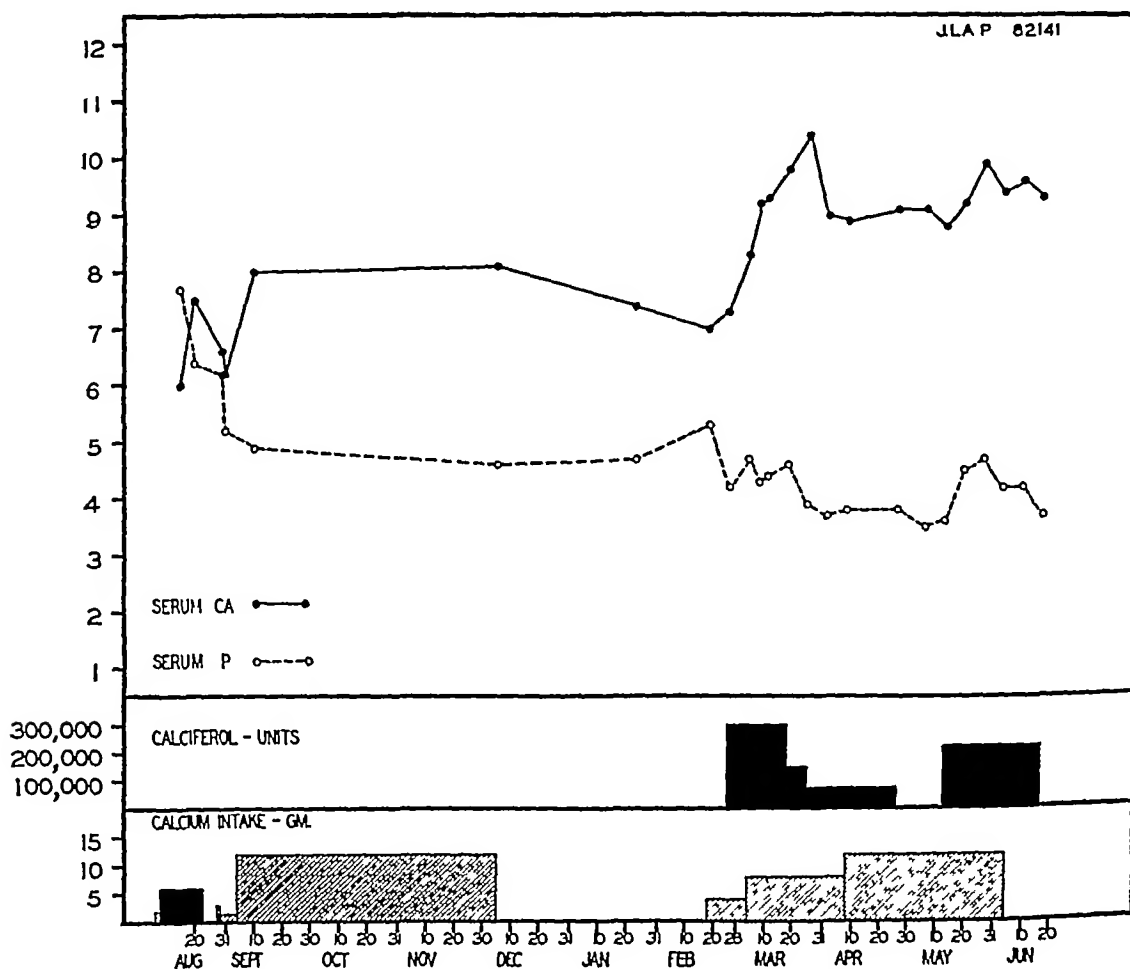
FIG 3 CASE II, J LA P IDIOPATHIC PARATHYROID TETANY

Calciferol 1 mgm equivalent to 40,000 international vitamin D units Calcium salts Solid blocking = calcium chloride, shaded blocking = calcium lactate
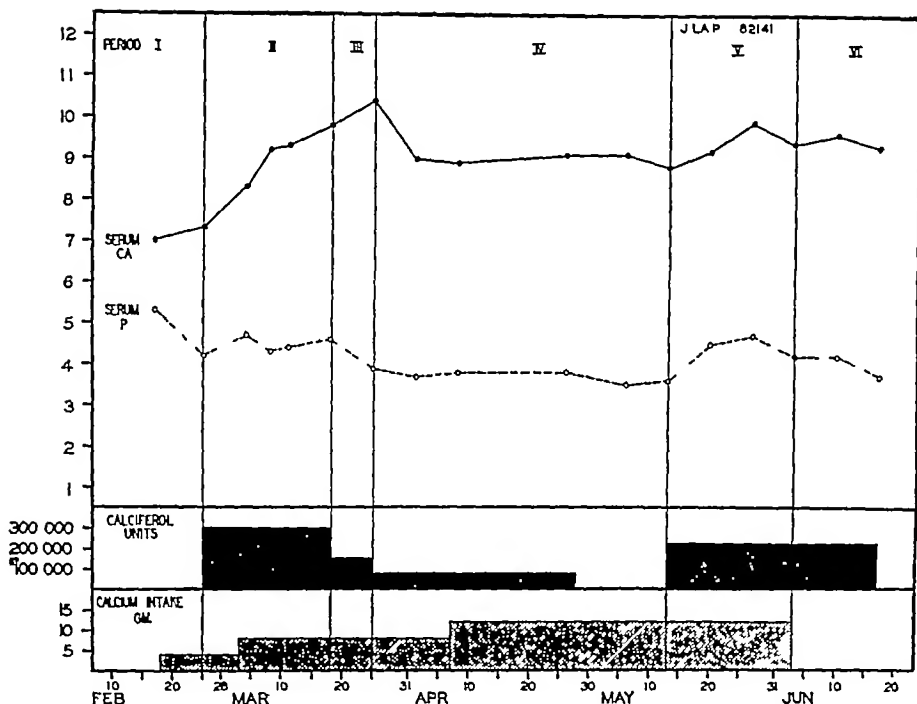
FIG 4   CASE II, J LA P   IDIOPATHIC PARATHYROID TETANY

Calciferol 1 mgm. equivalent to 40 000 international vitamin D units   Calcium salts   Solid blocking = calcium chloride   shaded blocking = calcium lactate

neutral acriflavine and alkali for a period of five months when all urinary symptoms disappeared. Although the urine no longer contained pus cells culture yielded *B coli* as late as March 1937

Period I (6½ months)   The initial attack of tetany (August 15 1936) was promptly relieved by the intra venous injection of 1 gram of calcium gluconate. Moderate amounts of calcium were then administered orally Although there was no further active tetany Trousseau s sign remained positive.   The patient was discharged from the hospital on August 22, 1936, without medication Six days later she was readmitted in acute tetany   This promptly responded to calcium therapy   The patient was discharged on September 5 1936 and advised to take 16 cc. of cod liver oil and 12 grams of calcium lactate daily Hypocalcemia and hyperphosphatemia persisted but mani fest tetany did not reappear until February 17 1937   The average serum values for the entire period were calcium 7 1 mgm. per cent   phosphorus 5 6 mgm. per cent.

Period II (21 days)   Doses of 300 000 vitamin D

units of calciferol with 4 and then 8 grams of calcium lactate were administered daily   The serum calcium rose steadily to 9.8 mgm. per cent but the serum phosphate remained constant between 4.2 and 4.7 mgm. per cent. Signs of latent tetany disappeared when the serum cal cium reached a level of 8.3 mgm. per cent.

Period III (7 days)   The daily calciferol intake was reduced to 150 000 units but the serum calcium continued to rise and the serum phosphate to fall reaching new levels of 10 4 and 3 9 mgm. per cent respectively

Period IV (49 days)   The daily calciferol intake was reduced still further (to 75 000 units) and the calcium intake increased to 12 grams of the lactate   Serum cal cium fell to and remained fairly constant at 9 mgm. per cent   The serum inorganic phosphorus fell to 3.5 mgm. per cent. At the end of four weeks calciferol admin istration was discontinued, but the serum calcium re mained elevated for the following three weeks

Period V (21 days)   Calciferol therapy was resumed at a somewhat higher level 225 000 units daily   There

was a gradual rise in the serum calcium to 9 9 mgm per cent and in the serum inorganic phosphorus to 4 7 mgm per cent

Period VI (14 days) Calciferol was administered as in Period V but calcium lactate was omitted The serum calcium remained at essentially the same level for two weeks, in contrast to the rise of serum calcium which followed the continued administration of comparable doses of calciferol supplemented by calcium lactate (Periods II, III, V)

Except for the lapse of three weeks in Period IV, calciferol was administered continuously for 106 days At no time were there any signs or symptoms of tetany, and the serum values for calcium and phosphorus remained within normal limits There was no evidence of hypervitaminosis D and the weight remained constant.

The patient ate a well balanced diet of her own choosing during the entire period of observation Its average daily calcium and phosphorus content was determined as for Case 1 and found to contain 0 434 gram and 0 829 gram respectively

DISCUSSION

The results of this study confirm Elliott's (19) and Stacey's (20) conclusion that crystalline vitamin D_2 (calciferol) is an effective agent in the treatment of parathyroid tetany Case 1 had suffered from severe tetany for at least one year preceding the use of calciferol and had failed to respond to parathormone, low phosphorus-high calcium diet, two parathyroid gland transplants, moderate doses of viosterol, and calcium salts in amounts suggested by Boothby and Davis (23) Large doses of calciferol, in the presence of an adequate calcium intake, maintained the patient in a normal state during the succeeding six months Case 2, on the other hand, had suffered from mild tetany for a period of six months preceding the use of calciferol Although moderate doses of calcium and cod liver oil prevented severe tetany, there were mild attacks at each menstrual period and hypocalcemia was constantly present By the use of calciferol and an adequate calcium intake, it was possible to maintain the patient in a normal state with respect to her tetany and blood chemistry during the succeeding four months It must be granted, however, that larger doses of calcium and viosterol, such as were used in Case 1, might have been equally effective

Serum calcium —The effect of calciferol on the serum calcium was found to be dependent on the daily dose, duration of dosage, and the calcium intake Moreover, there appeared to be individual differences between the two patients

Daily doses of 225,000 to 300,000 vitamin D units resulted in a progressive rise in the serum calcium in both patients, when the calcium intake was adequate Doses of 150,000 units daily resulted in a rise in Case 2 (Period III), but merely maintained the serum calcium at its initial level in Case 1 (Period IX) In Case 2 (Period V), 75,000 units daily were inadequate to maintain a serum calcium of 10 4 mgm per cent

When adequate amounts of calciferol were administered, there was an appreciable rise in the serum calcium at the end of a week, often at the end of three days A daily dose of 300,000 units of calciferol was ineffective when given for less than one week A similar dose given ten days later, however, resulted in a very definite rise in the serum calcium, apparent evidence of cumulative action (Case 1, Period III) The continued rise in the serum calcium after cessation of calciferol administration also suggested cumulative action (Case 1, Period II)

Calciferol was administered uninterruptedly to Case 1 for four months without the development of hypercalcemia Since an effort was made to maintain the serum calcium at the lower limits of normal, to avoid toxic manifestations, daily doses as large as 300,000 units were not administered for longer than three weeks, so that it is impossible to say whether the serum calcium would have continued to rise at the end of that time Crimm's work (24), however, on the effects of viosterol in tuberculous patients, seems to indicate that continued administration of large doses of vitamin D does result in hypercalcemia and a prolonged cumulative effect He administered the daily equivalent of 500,000 vitamin D units for eight to thirty-two days The serum calcium reached abnormal levels (13 8 to 18 5 mgm per cent) within five days, increased with the continued administration of viosterol, continued to rise after viosterol was discontinued, and remained abnormally high for as long as 213 days

When the calcium intake was reduced to 4 grams of the lactate in Case 1, the serum calcium fell sharply and the patient developed severe tetany, even though 300,000 units of calciferol were being administered daily (Period V) Similarly, in Case 2, daily administrations of 225,000 units of calciferol without added calcium salts failed to raise the serum calcium, even though

comparable doses supplemented by calcium lactate had previously effected very definite rises (compare Period VI with Periods III and V)

Bauer, Marble, and Claflin (4) were among the first to demonstrate the direct relationship between the calcium intake and the effectiveness of irradiated ergosterol in raising the serum calcium Five milligrams of irradiated ergosterol daily (equivalent to approximately 100,000 vitamin D units) raised the serum calcium to normal levels in a case of hypoparathyroidism, when the calcium intake was adequate, but was ineffective when the calcium intake was greatly reduced In experimental animals, on the other hand, Harris and Innes (25) and Shelling (2) were able to induce viosterol hypercalcemia in the absence of calcium from the diet The doses used, however, were tremendous and not comparable to those used in the present or other studies on humans

The failure of some workers (9 10) to demonstrate a rise in the serum calcium after the administration of vitamin D to parathyroprivic patients can probably be explained on the basis of inadequate dosage and the failure to include adequate available calcium in the diet

The larger calcium requirement of Case 1, as compared to Case 2, may have been related to the presence of hyperthyroidism, since, as Aub and his associates (26) have demonstrated there is an increased excretion of calcium in hyperthyroidism

Serum inorganic phosphorus —Shelling and his coworkers (11, 12) have questioned the advisability of using vitamin D in parathyroid tetany since it primarily increases the concentration of the inorganic phosphorus in the blood and favors phosphate retention Other investigators have not confirmed these findings Bauer Marble, and Claflin (4) found that a daily dose of 5 mgm. of irradiated ergosterol supplemented by a high calcium intake, resulted in a decreased serum phosphate and a negative phosphorus balance in a case of parathyroid tetany Similarly Crimm (24) demonstrated in tuberculous patients fed large doses of viosterol a transient rise followed by a sustained fall in the serum inorganic phosphorus This transient rise in the serum phosphate may be the explanation for Shelling and Goodman's controversial results (11) In their first patient the serum phosphate rose on the third day of viosterol

administration but had started to fall by the sixth day when viosterol administration was discontinued Had viosterol been continued there is reason to believe from Crimm's work that the serum phosphate would have fallen further and the serum calcium could have risen to normal levels

Although the effect of calciferol on the serum inorganic phosphorus was not as constant as on the serum calcium, there was, in general, a reciprocal relationship between the serum calcium and serum phosphate in the present study In both Cases 1 and 2, the serum phosphate was lowered to and remained at normal levels during the entire period of calciferol administration This is strikingly illustrated in the divergence of the serum calcium and phosphorus curves in Figures 1 and 3 Similarly, in the four cases of tetany treated with calciferol by Stacey (20) and Elliott (19), there was a reciprocal fall in the serum phosphate as the serum calcium rose

The diet is no less important in its effect on the *serum phosphate than it is on the serum calcium level* As has been demonstrated by Shelling and Goodman (11) and others (10 27), high phosphorus diets predispose to hyperphosphatemia whereas low phosphorus diets result in a reduction of the serum phosphate The use of high calcium-low phosphorus diets in parathyroid tetany is to be commended as a logical procedure Both subjects of this investigation were on a relatively low phosphorus (less than 1 0 gram) and high calcium intake. The diet undoubtedly played some role in lowering the serum phosphate but the major effect must have been due to vitamin D since the average serum phosphate level on the diet alone was 4.9 mgm per cent in Case 1 and 5 6 mgm per cent in Case 2 whereas the averages for the calciferol periods were 2 8 mgm per cent and 4 1 mgm. per cent respectively

Mode of vitamin D action —Since they were able to protect monkeys against dietary tetany with irradiated ergosterol when the parathyroids were intact but not after they had been removed Hess and Lewis (28) concluded that irradiated ergosterol acted by stimulating the parathyroids Greenwald and Gross (29) came to a similar conclusion and suggested the presence of accessory parathyroid tissue to explain the activity of vitamin D in parathyroidectomized animals

Bauer and his associates (4, 7) and others (30), on the other hand, have refuted the " parathyroid theory" of vitamin D action and have demonstrated that, both in normals and patients suffering from hypoparathyroidism, the essential action of irradiated ergosterol is to increase absorption of calcium and phosphorus from the intestine In normals, the excess calcium and phosphorus absorbed is rapidly excreted so that there is very little change in the lime salt balance (7) Where there is a deficiency of calcium and phosphorus in the serum and bones, as in osteoporosis and osteomalacia, the extra calcium and phosphorus absorbed are retained in the serum and then deposited in the bones (31) In hypoparathyroidism the action of vitamin D appears to be somewhat different There is an increased absorption of calcium from the intestine, some retention in the serum, and an increased excretion in the urine Phosphorus, on the other hand, appears to be excreted in increased quantity through the bowel, while the serum content falls (4) This apparent loss of phosphorus in the feces does not preclude, however, the possibility of increased absorption, since, as Shelling (32) has pointed out, the excretion of phosphorus through the bowel is an important mechanism when the kidneys are no longer able to handle an excess

Although the theory that vitamin D increases the absorption of calcium and phosphorus from the gut explains the effects of moderate doses, it cannot explain the development of the hypercalcemia and negative calcium balance that occurs when large doses of viosterol are administered to animals on calcium-free diets (2) Taylor and his associates (33) feel that excessive doses reverse the usual effects of viosterol, resulting in decalcification of the bones and a negative calcium balance, by stimulation of the parathyroids Shelling (12) has analyzed Taylor's experiments and pointed out that they " neither prove nor disprove that the parathyroids regulate the activity of vitamin D "

The present study of pure crystalline vitamin D$_2$ (calciferol) adds but little to the solution of this problem, since calcium and phosphorus balance studies were not carried out In general, the response was similar to that seen with crude irradiated ergosterol in parathyroid tetany (4) The dependence of calciferol on an adequate cal-

cium intake in raising the serum calcium, suggested increased absorption or possibly decreased excretion in the gut The reduction of phosphatemia could have been due to deposition of phosphorus in the bones, increased excretion into the intestine, or increased phosphaturia In the light of Bauer's work (4) on irradiated ergosterol, the last would appear to be the most likely

Crimm and Strayer (34), using doses of viosterol large enough to induce hypercalcemia in tuberculous patients, noted an increase in the serum total proteins, the albumin fraction and the pH, and a decrease in the globulin fraction and the chlorides The carbon dioxide content remained constant In the present study no significant changes were noted in the concentrations in the serum of any of these substances

Toxicity of vitamin D —Excessive doses of irradiated ergosterol have been shown to be toxic for man (35) and experimental animals (6, 8, 25) Shohl, Goldblatt, and Brown (16) found that rats fed 4 mgm of irradiated ergosterol (Vigantol) daily, lost weight and died in from 5 to 14 days with hypercalcemia, decalcification of the bones, parenchymatous lesions and metastatic calcification of the kidneys, heart, blood vessels, and gastric mucosa In two infants who died of hypervitaminosis D, Thatcher (36) described pathological calcification and cellular infiltration of the kidneys, fatty degeneration of the liver, but no calcification of the blood vessels or soft tissues That toxic manifestations may occur without pathological calcifications has been demonstrated by Shelling (32) and others (25) The arterial calcifications seen in experimental hypervitaminosis D do not appear to be related to the arteriosclerosis of man, but represent a deposition of calcium limited to the media, similar to that seen in Monckeberg's sclerosis (8)

The pathogenesis of metastatic calcification is unknown, but it appears to be related to the presence of hypercalcemia and/or hyperphosphatemia (30) Ham and Portuondo (37), however, were not able to demonstrate a direct relationship between the level of the serum calcium and pathological calcification, and suggested that the precipitation of calcium in the tissues was due to a change in the state of the serum calcium rather than its level Smith and Elvove (6) believed that hyperphosphatemia was the determining fac-

tor, since they found that hyperphosphatemia, in the presence of even a slight increase in the serum calcium, resulted in metastatic calcification, while hypercalcemia in the presence of a normal or low serum phosphate never gave rise to abnormal calcifications That the phosphatase content of the tissues may play a role in the deposition of calcium has been suggested by Harris (30), on the basis of Martland and Robinson's studies on ossification (38)

The toxicity of vitamin D is dependent, at least in part, on the diet and renal function (8, 39) High phosphorus intake and renal impairment, which result in phosphate retention, predispose to greater metastatic calcification

The toxic symptoms of viosterol overdosage in man have been best described by Reed (35), who studied the effects of tremendous doses in patients with hay fever and related conditions Of the 300 patients studied, 43 developed one or more of the following symptoms urinary frequency, anorexia, nausea, vomiting diarrhea, loss of weight, muscle weakness, muscular incoordination and disturbed equilibrium Reed feels, however, that "there need be little apprehension about the administration of amounts up to 150,000 international units daily for indefinite periods" and that doses equivalent to those given dogs to produce medial sclerosis have never been given to man In the elderly patients he studied, there did not appear to be any evidence of increased arterio sclerosis or hypertension Crimm (24) was unable to demonstrate any evidence of decalcification, such as is seen in experimental animals, in patients with hypercalcemia of long duration

Many of the studies on hypervitaminosis are open to question since the vitamin products used (chiefly Vigantol) have been shown to contain toxisterol (13) That vitamin D itself, however, is toxic when given in large enough amounts, is borne out by the fact that the pure crystalline vitamin D₂ (calciferol) has been shown to be toxic for experimental animals (17) and man (20)

The two subjects of this report exhibited none of the usual symptoms of vitamin D toxicity during the administration of calciferol. There was no direct evidence of metastatic calcification and, since the serum calcium and serum phosphate were kept within normal limits, there was no rea-

son to suspect that it occurred The mild degree of decalcification of the bones in Case 1 was probably related to the long standing state of hyperthyroidism, and the diminished renal function in Case 2 can probably be attributed to the presence of pyelitis

Advantages of calciferol over parathormone in chronic tetany —The transient effect, the necessity for repeated subcutaneous injections, and the tendency toward a negative calcium balance, and the possible deleterious effects on the remaining parathyroid tissue (12) make the prolonged use of parathormone in chronic tetany objectionable Calciferol, on the other hand, has a prolonged effect, may be given orally, tends to increase calcium retention and if Shelling's theory (12) is correct, may lead to a hypertrophy of the remaining parathyroid tissue The objection raised by Shelling and Goodman (11, 12) that vitamin D raises the serum phosphate in addition to the calcium and is, therefore, not effective, has been shown to be invalid The necessity for frequent determinations of serum calcium and phosphate, to control dosage, is equally true of parathormone and calciferol The danger of toxic effects did not appear to be any greater with calciferol than with parathormone administration

Since calciferol is relatively slow in its action, there appears to be a place for parathormone therapy where a rapid rise in serum calcium is desired Thereafter, the use of calciferol would appear to be advantageous Calciferol is not advocated as a universal therapeutic agent in tetany but is recommended in those cases of chronic parathyroid tetany which do not respond to high calcium low phosphorus diets, and in which satisfactory parathyroid gland transplants are not possible.

SUMMARY AND CONCLUSIONS

1 Two cases of chronic parathyroid tetany were presented which failed to respond to the usual therapeutic agents used in such cases

2 Calciferol (crystalline vitamin D₂) satisfactorily controlled the tetany and maintained the serum calcium and phosphate at normal levels in both patients for periods of four and six months respectively

3 The action of calciferol appeared to be de pendent upon an adequate calcium intake.

4 No toxic manifestations were demonstrable with the doses of calciferol used

5 Calciferol is recommended as a valuable therapeutic agent in cases of chronic parathyroid tetany which do not respond to high calcium-low phosphorus diets and in whom satisfactory transplants of parathyroid gland are not possible

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TABLE I

The absence of sulf- and methemoglobinemia in patients showing cyanosis after sulfanilamide treatment

Date	Diagnosis	Cyanosis	Hemoglobin	Blood Iron	Calculated CO capacity	Measured CO capacity	Possible sulf- and methemoglobin
1937			*grams per 100 ml*	*mgm per 100 ml*	*volumes per cent*	*volumes per cent*	
April 22	Puerperal sepsis	+	9 13	30.50	12 20	12.20	None
May 11	Puerperal sepsis	+	0.24	30 95	12 38	12.39	None
May 17	Puerperal sepsis	+	6 10	20 4	8 16	8 18	None
July 3	Septicemia after hand infection	++	12.65	42 4	16 96	16 90	Negligible
August 21	?	++	11 13	37 4	14 96	14 88	Negligible
October 1	Ear and brain abscess	+	7 86	26.3	10.52	10.32	Negligible
October 13	Scarlet fever	++	7 42	24 8	9 92	9 81	Negligible
1938							
February 23	Puerperal sepsis	++	9 06	30.3	12.12	12 06	Negligible

sulfanilamide were always between 5 and 10 mgm per cent, when determined In these control experiments, therefore, concentrations of this order were maintained As the data in Table II show, sulfanilamide has no effect upon the CO capacity of either met- or sulfhemoglobin

TABLE II

The failure of sulfanilamide to affect the CO capacity of met- and sulfhemoglobin

Methemoglobin

	Hemoglobin plus methemoglobin	Active hemoglobin	Sulfanilamide			
			5 mgm per cent	10 mgm per cent	20 mgm per cent	40 mgm per cent
CO capacity, *volumes per cent*	13 85 (Hyposulphite)	6 72	6 82	7 05	6 68	6 82

Sulfhemoglobin

	Hemoglobin plus sulfhemoglobin	Active hemoglobin	Sulfanilamide			
			5 mgm per cent	10 mgm per cent	20 mgm per cent	40 mgm per cent
CO capacity, *volumes per cent*	16 93 (Iron)	12 22	12 10	12 10	12 22	12 22

Marshall and Walzl (4) found only one patient in seven (five of whom were cyanotic), in whom there was any methemoglobin This patient had only a small amount of methemoglobinemia which almost certainly could not alone have caused the

cyanosis In the one case reported by Mull and Smith (5), there is the possibility that sulf- or methemoglobin existed However, the oxygen unsaturation which they observed was perhaps sufficient to cause cyanosis from reduced hemoglobin alone, there being as high as eight grams of the reduced form

This observation of Mull and Smith is well worth checking in a series of patients, for if such degrees of oxygen unsaturation do exist, the sulfanilamide is producing an anoxia The frequent reactions to sulfanilamide such as headache, nausea, malaise, vertigo, lassitude, tinnitus, accelerated pulse and respiration, and loss of alkali in the urine constitute the syndrome of " mountain sickness " which is caused by an anoxic anoxia If the drug does have this effect, perhaps the customary use of sodium bicarbonate with sulfanilamide is not wholly desirable The excretion of alkali with the fall in blood CO_2 combining power would appear to be a compensatory response to the loss of carbon dioxide blown off during the accelerated respiration Also sulfanilamide has been recommended in treating pneumonia In pneumonia, anoxia is often already a serious complication

The fact that Marshall and Walzl (4) with 7 cases, Mull and Smith (5) in one case, and the writer, 8 cases, have not found sulf- or methemoglobinemia in high enough concentration to cause noticeable cyanosis casts serious doubt upon the prevalent idea that cyanosis following sulfanilamide is usually attributable to these hemoglobin derivatives This is not to deny that in some cases the cyanosis may be so caused Discombe (2) did find considerable sulfhemoglobinemia in six of seven cases He has attributed this to the concurrent use of magnesium sulphate which presumably causes an enterogenous sulfhemoglobinemia perhaps catalyzed by sulfanilamide Paton and Eaton (3) found four out of nineteen patients to have a methemoglobinemia, which was proved spectroscopically In the present series, no patient was allowed saline cathartics, which might account for the absence of sulfhemoglobin

From this we can only conclude that before attributing the cyanosis in a given case to sulf- or methemoglobinemia, these pigments must be identified, and shown to be present in such concentra-

tion as would give the observed degree of cya
nosis

What the actual mechanism of the usual cyano-
sis is one cannot say   Marshall and Walzl (4)
suggested that a black oxidation product of sulf-
anilamide might possibly stain the erythrocytes
Perhaps, as Mull and Smith's data suggest, re-
duced hemoglobin is responsible   This would pre-
clude cyanosis in severely anemic patients

SUMMARY

Sulf- and methemoglobinemia were ruled out
in eight patients showing cyanosis after sulfanila-
mide therapy   The theoretical carbon monoxide
capacities calculated from the blood irons, and
the measured CO capacities check closely   There-
fore all hemoglobin is present in an active form

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one and one-half minutes after the response to the first test had been elicited The time was recorded from the beginning of the injection until the patient perceived the bitter taste The injection time was also recorded, but since the response may come with a minimal amount of the drug, the time which we have used was taken from the start, rather than from the conclusion of the injection

The venous pressure was measured by the direct method (13), using a large antecubital vein, the arm being placed on a level with the right auricle. The apparatus consisted of an L-tube of glass attached to a three-way stopcock, a syringe, and an 18-gauge needle The apparatus was filled with a solution of sterile normal saline, a venepuncture performed, and the direct pressure readings recorded Normal pressures with this apparatus range from 4 0 to 9 0 cm of saline The antecubital vein of one arm was reserved for the injection of decholin and of the other arm for the measurement of venous pressure In subsequent measurements the vein was entered at the site first punctured

X-ray photographs of the heart were taken with the patient in the standing position, in full inspiration, at a distance of two meters[2] Measurements of the cardiac area were carried out by the technique of Levy (14) and estimations of volume were made as recommended by Bardeen (15) The volumes recorded in Table I have not been multiplied by the constant which is in Bardeen's formula This was done in order to make our observations comparable to those of Starr and his coworkers (7, 8) The patients assumed as nearly as possible exactly the same position for each observation in order to make comparison with subsequent ones of this patient possible and to assure uniformity from this point of view In addition, each procedure in the observation was carried out by the same investigator

Observations were made first during the abnormal rhythm and later again after restoration of normal sinus rhythm[3] Most of the patients exhibited no clinical evidence of congestive heart failure (see text), nor were they anemic (16)

PART I OBSERVATIONS RELATING TO RHYTHMS ASSOCIATED WITH RAPID VENTRICULAR RATE

OBSERVATIONS RELATING TO AURICULAR FIBRILLATION

In Table I are reported seven patients exhibiting auricular fibrillation In them, observations were made first during auricular fibrillation and

later again after restoration of normal sinus rhythm

R D (N Y H No 48714), a white man, aged 32 years, was admitted to the hospital on December 6, 1933, complaining of sudden onset of irregularity of the heart beat the day before while "cranking an automobile." The first attack occurred 11 years before when he was 21 years old He had experienced approximately one attack every 6 months since then Each attack was of 5 to 6 days' duration Neither digitalis nor quinidine had been effective in terminating an attack He had never exhibited manifestations of rheumatic fever When seen 24 hours after the onset of this attack he did not appear acutely ill, there were no signs of congestive heart failure The heart was not enlarged The diagnoses were *A*, unknown , *B*, mitral stenosis , *C*, paroxysmal auricular fibrillation[4] On December 8, 1933, when auricular fibrillation was present, measurements of the circulation were made, these were repeated on December 11th, 2 days after restoration of normal sinus mechanism occurred spontaneously (Table I, Figure 1) In October 1936 we had occasion to make observations of this patient during still another attack of paroxysmal auricular fibrillation Following discharge from the hospital in 1933 he experienced attacks of paroxysmal auricular fibrillation at 6 to 7-month intervals, during the last year, however, the frequency had increased to 3 attacks This last attack began on October 25, 1936 Because of its persistence the patient was admitted to the hospital on October 27, 1936 The physical signs were essentially the same as on the first admission, as before there were no signs of congestive heart failure, and there were signs of early mitral stenosis Studies of the circulation were made on October 28, 1936 in the presence of auricular fibrillation and again on October 30, 1936, 24 hours after reversion to normal sinus mechanism under quinidine therapy (Table I, Figure 1) Measurements of the circulation during normal sinus rhythm in 1934 were approximately the same as two years later in 1936 during this rhythm, and no significant change in the size of the heart had occurred during this interval

J R (N Y H No 52524), a white man, aged 21 years, was admitted to the hospital on January 17, 1934, complaining of rapid, irregular, forcible beating of his heart of 16 hours' duration He had never experienced a similar attack History of rheumatic infection was not elicited Examination revealed no evidence of congestive heart failure After exercise a presystolic impurity of the first sound was heard at the apex, this was not sufficient, however, to warrant the diagnosis of valvular disease. The heart was not enlarged The diagnosis was

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[2] The authors are deeply indebted to the X-ray Department of the New York Hospital for their cooperation in this investigation

[3] Deviation from this routine is indicated in the text and tables

[4] The diagnoses in this paper conform to the nomenclature for cardiac diagnosis recommended by the Heart Committee of the New York Tuberculosis and Health Association " Criteria for the Classification and Diagnosis of Heart Disease " New York Tuberculosis and Health Association, New York, 1929, 2d ed

TABLE I

Data on patients exhibiting auricular fibrillation auricular flutter and paroxysmal tachycardia

Auricular fibrillation → Normal rhythm without digitalis without quinidine (Figure 2)

Auricular fibrillation → Normal rhythm with quinidine without digitalis (Figure 1)

Auricular fibrillation with digitalis with quinidine → Normal rhythm (Figure 1)

Auricular fibrillation with digitalis → Normal rhythm (Figure 1)

Auricular fibrillation with digitalis with quinidine → Normal rhythm (Figure 1)

grams[5] in 24 hours On October 27th when the heart rate had decreased to 92 per minute, studies were repeated Maintenance doses of digitalis, 0 2 gram, were now given daily and the use of quinidine was instituted On the 10th day of its administration (total given 9 7 grams), reversion to normal sinus rhythm occurred At this time, November 8, 1934, measurements of the circulation were repeated, the patient still being under the influence of digitalis When auricular fibrillation recurred one week later, no further effort was made to restore the rhythm to normal sinus mechanism

F K (*N Y H No 90264*), a white male, aged 61 years, was admitted to the hospital February 27, 1935 A history of rheumatic fever was not secured Syphilitic infection was denied He had been well until the onset of the present illness Five days before admission he began to expectorate frothy white sputum associated with hoarseness and wheezing respirations Three days before admission, he experienced a sense of suffocation for 5 minutes following which he became dyspneic on exertion On admission dyspnea, cyanosis, basal pulmonary râles, and slight edema were evidence of congestive heart failure Auricular fibrillation with rapid ventricular rate was present. The heart was enlarged and x-ray examination revealed aneurysm of the ascending aorta The Wassermann reaction of the blood was positive The diagnoses were *A*, lues, *B*, aneurysm of the ascending aorta, cardiac enlargement, *C*, paroxysmal auricular fibrillation On February 28, 1935, when the ventricular rate was 119 per minute, observations were made (Table I, Figure 1) The next day digitalis 2 0 grams was given The day following (March 2, 1935), when the ventricular rate was 90 per minute, observations were repeated On March 4, 1935, spontaneous reversion to normal sinus rhythm occurred and on March 6, 1935, the rhythm being normal and the patient still under the influence of digitalis, studies were carried out again On March 7, auricular fibrillation recurred and persisted

P B (*N Y H No 57869*), a white male, aged 55 years, was admitted to the hospital on April 10, 1934, because of urethral stricture History of rheumatic infection was not obtained For 10 years he had experienced attacks of irregularity of the heart associated with forceful heart beat and sensation of palpitation The attacks were sudden in onset and in offset During passage of a urethral sound the patient complained of "fluttering" of the heart Auricular fibrillation was found to be present The heart was slightly enlarged, there was no valvular defect The diagnoses were *A*, arteriosclerotic heart disease, *B*, cardiac enlargement, *C*, paroxysmal auricular fibrillation He was given digitalis On April 13, 1934, when the ventricular rate was

98 per minute, and there were no signs of congestive heart failure, observations were made (Table I, Figure 1) On giving quinidine, reversion to normal sinus rhythm occurred, and on April 20th, 24 hours afterward, studies of the circulation were repeated

W H (*N Y H No 64020*), a white male, aged 24 years, was admitted to the hospital September 16, 1935, because of mild recurrence of rheumatic infection. He was discharged December 23, 1935 He had suffered from chorea since he was 8 or 10 years of age. At 10 years of age rheumatic heart disease was discovered Mitral stenosis and insufficiency had been present since 12 years of age. The heart was enlarged. Normal sinus rhythm was present The diagnoses were *A*, rheumatic fever, *B*, mitral stenosis and insufficiency, cardiac enlargement, *C*, paroxysmal auricular fibrillation. On October 7, 1935, when the patient was under the influence of digitalis and there were no signs of congestive heart failure, observations of the circulation were made (Table I, Figure 1) On November 8, 1935, the rhythm changed to auricular fibrillation On November 20, 1935, when auricular fibrillation was present, the patient still being under digitalis, studies were made again (Table I, Figure 1)

Summary of effects of auricular fibrillation

1 In 7 patients, the cardiac output in all except one (C C) was less during auricular fibrillation than after the reversion to normal sinus rhythm, whether the patient was or was not under the influence of digitalis at the time reversion to normal sinus rhythm occurred In one patient (M R) on each reversion from normal rhythm to auricular fibrillation, the cardiac output decreased, and increased again when the normal rhythm supervened In two instances (C C and F K) when digitalis was given during auricular fibrillation there resulted increase in cardiac output and decrease in cardiac size,[6] in one of these (F K) reversion to normal mechanism was accompanied by still further increase in output to normal limits

2 The heart was in some instances larger in the presence of auricular fibrillation than after restoration of normal sinus rhythm, and in other instances it was unchanged When the use of digitalis was resorted to, decrease in size of the heart was observed Results similar to this are reported by Stewart and his coworkers (18, 19, 20)

3 In those instances in which the arm to tongue circulation time was measured, it was prolonged

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[5] Experience with this particular "batch" showed that this amount was required regardless of body weight to slow the rapid ventricular rate in the presence of auricular fibrillation to around 70 per minute, when given within 24 hours, it was considered the digitalizing amount (17)

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[6] These observations are being reported at greater length (18, 19)

during auricular fibrillation and became shorter on restoration of normal sinus rhythm

4 The venous pressure was elevated in those instances in which it was measured and restoration of normal sinus rhythm witnessed a fall to normal levels

OBSERVATIONS RELATING TO AURICULAR FLUTTER

There are observations of 4 patients exhibiting auricular flutter (Table I) In two of these (Cases H B and G G) comparison could be made between those made during auricular flutter and during normal sinus rhythm

H B (N Y H No 113637) a white male, aged 21 years was admitted to the hospital on November 25 1935 Eleven days before admission he experienced shortness of breath, weakness fatigue, and a tight feel ing under the sternum on running up elevated railway stairs. Three days before admission he vomited and on examination by his physician paroxysmal tachycardia was discovered. The past history was not important. On examination there was slight cyanosis of the lips and the liver was palpable. The electrocardiogram showed 2 1 *auricular flutter There was no evidence of valvular disease.* The diagnoses were *A* unknown, *B* cardiac dilatation *C* paroxysmal auricular flutter

Measurements were made on November 26 1935 when auricular flutter was present (Table I, Figure 1) Digitalis, 2.2 grams was given in 28 hours, and measurements were repeated on November 29th reversion to normal sinus rhythm having occurred the day before. More digitalis was not given and observations were made again on December 2d, as well as on December 18th, 21 days after digitalis had been discontinued. To make a valid comparison of the effect of auricular flutter on the circulation in this patient, the first observations made during auricular flutter (November 26th) and the last ones (December 18th) made after excreting digitalis should be compared (Table I Figure 1) In this patient the presence of auricular flutter was not associated with rise in venous pressure.

G G (N Y H No 108714) a white male aged 56 years, was admitted to the hospital on September 13 1935 The patient gave a history of attacks of paroxysmal tachycardia during the last 14 years The recent attack began 5 days before admission and was associated with headache, nervousness, constriction of the chest weakness palpitation and vomiting Dyspnea was present as well as cyanosis pulmonary rales and enlargement of the liver The electrocardiogram showed 2 1 auricular flutter auricular rate 216 per minute. There was no evidence of valvular disease. The diagnoses were *A* arteriosclerosis *B* coronary artery disease cardiac enlargement coronary occlusion [†] *C* paroxysmal auricular flutter

[†] This diagnosis was not made until after the patient had been under observation for some time.

lar flutter On September 14 1935, studies of the circulation were made (Table I, Figure 1) After administration of digitalis 0.8 gram, reversion to normal sinus rhythm occurred he received, however, in all a total of 1.2 grams. On September 16 1935, normal rhythm being present, observations were repeated as well as on September 20, 1935 The abnormal rhythm in this patient was associated with rise in venous pressure (see also observations relating to ventricular paroxysmal tachycardia) It was not until the digitalis effect on the electrocardiogram had worn off that it was suspected that this attack had been associated with occlusion of a [?] nary artery (see observations relating to ventricular paroxysmal tachycardia)

A G (N Y H No 94972) r white male, aged 34 years, was admitted to the hospital on April 19, 1935, because of tachycardia of one day's duration. A similar attack had occurred 3 months before. Valvular heart disease had been discovered one year earlier Eight months before admission dyspnea appeared since it progressed rapidly it required him to remain in bed. The heart was tremendously enlarged. There was cyanosis of the lips and the liver was palpable. The diagnoses were *A* unknown *B* mitral stenosis and insufficiency, aortic stenosis and insufficiency cardiac enlargement *C* paroxysmal auricular flutter On April 20 1935, studies of the circulation were made (Table I Figure 1) The patient was given digitalis and in 6 days received 4.5 grams when normal rhythm was restored Three hours later pulmonary edema developed and the patient died shortly afterward.

A GI (N Y H No 108422) a white female, aged 49 years was admitted to the hospital on September 25 1935 for measurements of the circulation. She was discharged the next day Auricular flutter with a slow ventricular rate was discovered one and a half years ago when the patient began to have dyspnea nervousness and loss of weight. A diagnosis of hyperthyroidism was made. After thyroidectomy had been performed, auricular flutter persisted although she was given quinidine, as well as digitalis She had experienced no signs of congestive heart failure and did not exhibit any at this time. The heart was perhaps slightly enlarged. In this patient the functions of the circulation which were measured were all in normal range while the patient was at rest and when the ventricular rate was slow (Table I Figure 1)

Summary of effects of auricular flutter

It appears that in auricular flutter with rapid ventricular rate there is marked decrease in cardiac output per minute and very marked decrease per beat, with prolongation of circulation time and dilatation of the heart. Increase in venous pressure occurred in certain ones and not in others With restoration of normal sinus rhythm, changes in all these functions in the reverse di-

rection toward a normal level occur In one patient who exhibited auricular flutter with a moderately slow ventricular rate approximately normal values were observed

OBSERVATIONS RELATING TO SUPRAVENTRICULAR PAROXYSMAL TACHYCARDIA

There are observations of three patients exhibiting paroxysmal tachycardia of the supraventricular type (Table I)

M H (N Y H No 85620) exhibited paroxysmal tachycardia of nodal origin She was a white female, aged 65 years, who had been subject to attacks of rapid, regular forcible beating of the heart associated with dyspnea and weakness since she was 37 years, of age They occurred every one to two months, lasting 5 minutes to 12 hours There were no known precipitating factors The attacks terminated spontaneously She was admitted to the hospital on January 16, 1935, during an attack of paroxysmal tachycardia, which was auriculoventricular in origin Reversion to normal sinus rhythm occurred before studies of the circulation could be made. The heart was not enlarged. The radial vessels were moderately thickened. The diagnoses were A, arteriosclerosis, B, no cardiac enlargement, C, auriculoventricular paroxysmal tachycardia On the morning of January 17th, the patient had only begun to eat breakfast when paroxysmal tachycardia recurred She remained quiet for several hours after which studies of the circulation were made (Table I, Figure 2) Shortly after finishing these observations normal rhythm recurred spontaneously, and observations were repeated. Between this time and discharge on January 19, 1935, the patient experienced two more paroxysms

C F (N Y H No 80473), a white female, aged 53 years, gave a history of having occasional attacks of rapid, regular beating of the heart, sudden in onset and offset for 15 years Dyspnea and weakness accompanied the attacks which were likely to be brought on by emotional disturbances She was admitted to the surgical service of the hospital on November 29, 1934, because of carcinoma of the rectum On December 5, 1934, an abdominal exploration and transverse colostomy was performed in preparation for removal of the carcinoma by the perineal route There was no evidence of valvular disease on examination The diagnoses were A, arteriosclerosis, B, no enlargement of the heart, C, auriculoventricular paroxysmal tachycardia. On December 20, 1934, at 3 p m., paroxysmal tachycardia arising above the ventricles occurred when the patient was told a second operation was to be performed Neither ocular nor vagal pressure was successful in ending the attack At 4 30 p m, morphine, 10 mgm, was given In the evening (11 p m), when the heart rate was 187 per minute, observations of the circulation were made (Table I, Figure 2) Following the injection of mecholin, 25 mgm, subcutaneously, reversion to normal sinus rhythm occurred
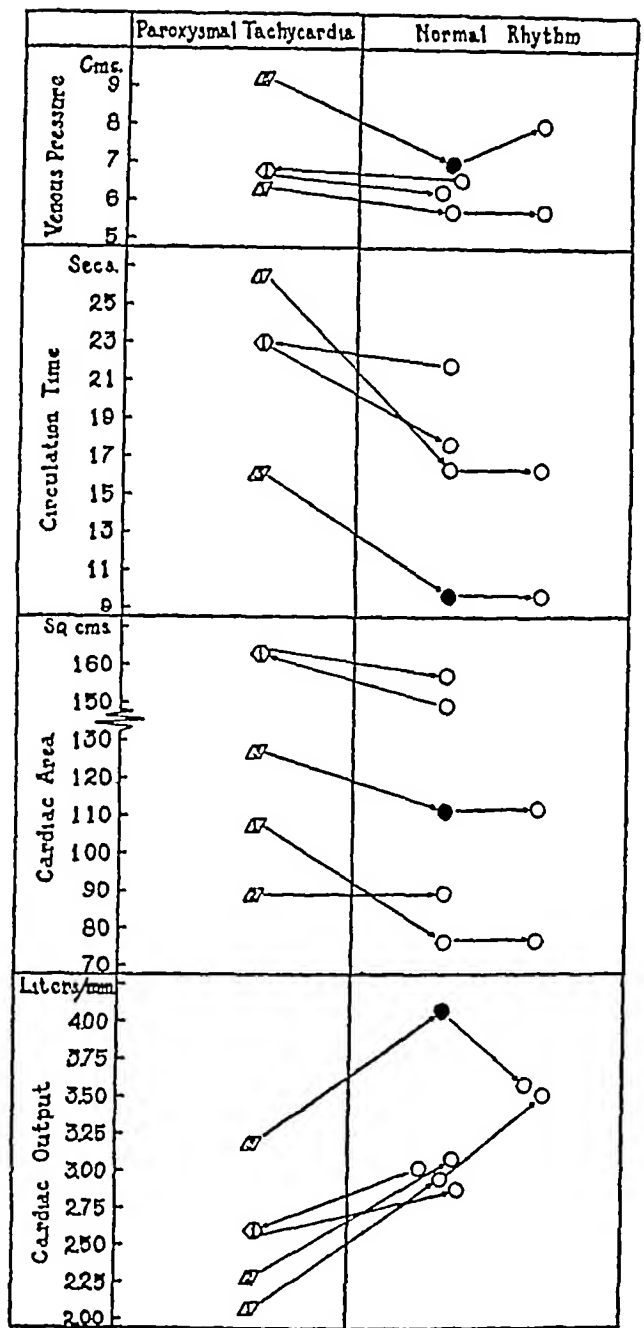
FIG 2 DATA RELATING TO STUDIES OF THE CIRCULATION IN PATIENTS EXHIBITING PAROXYSMAL TACHYCARDIA

In this figure as well as in Figure 3 parallelograms and hexagons and circles indicate that the patient exhibited supraventricular and ventricular paroxysmal tachycardia, and normal rhythm respectively The numerals 1, 2 and 3 in the parallelograms refer to Cases M H, C F, and R. T, respectively, and number 1 in the hexagon to Case G G, in Table I Closed and open symbols indicate that the patient was or was not under the influence of digitalis respectively

in four and a half minutes Forty five minutes later when normal sinus rhythm was present, studies of the circulation were repeated (Table I Figure 2)

The case of *R T* (*N Y H No 124470*) a white female, aged 29 years, illustrates the effect of auricular paroxysmal tachycardia. An attack started 4 days before admission, continuing for half a day Tachycardia recurred the day before admission. It was accompanied by dyspnea and anorexia. The patient suffered rheumatic fever at 11 years of age and was told she had valvular heart disease. She had indulged in ordinary activities since 11 years of age without discomfort. Since 15 years of age she had experienced attacks of paroxysmal tachycardia lasting from a few minutes to several hours Spontaneous reversion to normal sinus rhythm had occurred until this occasion. The patient exhibited no signs of congestive heart failure. The diagnoses were *A*, rheumatic fever *B* mitral stenosis and insufficiency *C* auricular paroxysmal tachycardia Studies of the circulation were made on February 26 1936 during auricular paroxysmal tachycardia (Table I Figure 2) The use of mecholin, 25 mgm. subcutaneously on two occasions resulted in reversion to normal sinus rhythm for a few minutes only Digitalis was then given and reversion to normal sinus rhythm occurred after administration of 1.6 grams. On February 28 1936 when the rhythm was normal studies were repeated as well as on March 14 1936 (2 weeks later) after excretion of digitalis The precise effect of the paroxysmal tachycardia in this patient is revealed by comparison of the data made during the paroxysm (February 26 1936) with those made during the normal sinus rhythm on March 14, 1936 after excretion of digitalis.

Summary of data relating to supraventricular paroxysmal tachycardia

It appears from studies of three patients exhibiting paroxysmal tachycardia of supraventricular origin that this rhythm was associated with decrease in cardiac output per minute and per beat and slowing of the circulation time. Rise in venous pressure did not occur

VENTRICULAR PAROXYSMAL TACHYCARDIA

There are studies of one patient suffering from ventricular paroxysmal tachycardia (Table I)

The history of *G G* (*N Y H No 108714*) has already been recorded (G G auricular flutter Table I) While the patient was still resting in bed, he suffered a second attack of paroxysmal tachycardia on September 26 1935 which was ventricular in origin at a rate of 179 per minute. This stopped spontaneously in 24 hours. The electrocardiogram after reversion to normal sinus rhythm suggested that this attack was associated with coronary occlusion On November 6, 1935 before allowing the patient to sit up studies of the circulation

were made. At this time, when normal sinus rhythm was present measurements of the circulation were made (Table I, Figure 2) On November 8th, ventricular paroxysmal tachycardia recurred, it was still present on November 10th, 36 hours later, at a ventricular rate of 200 per minute, and observations were repeated. The patient received 5.2 grams of quinidine in 3 days and reversion to normal sinus rhythm occurred at 2 a.m. on November 13th, and later in the morning when normal sinus rhythm was still present observations were repeated The patient experienced another attack of ventricular paroxysmal tachycardia on December 7th. He was discharged December 21, 1935

Summary of effects of ventricular paroxysmal tachycardia

Observations made of this patient before the onset of ventricular paroxysmal tachycardia, during paroxysmal tachycardia, and again after restoration of normal sinus rhythm revealed decrease in cardiac output per minute and per beat, prolongation of the circulation time, and dilatation of the heart during this rhythm.

PART II OBSERVATIONS RELATING TO RHYTHMS ASSOCIATED WITH SLOW VENTRICULAR RATE

There are eight patients exhibiting certain abnormalities of the rhythm in which the ventricular rate was slow in 4 (Cases H B , G N , A R., and C K.) complete heart block was present, in 2 (Cases T C. and J L.) 2 1 heart block, in 1 (Case H J) sinus bradycardia, and in 1 (Case J G) coupled rhythm due to auricular premature contractions (Table II)

OBSERVATIONS IN COMPLETE HEART BLOCK

There are observations of 4 patients exhibiting complete heart block (H B , G N , A R., and C K., Table II)

H B (*N Y H No 26415*) a white male, aged 56 years, was admitted to the hospital on March 22, 1934 He suffered an attack of acute rheumatic fever when 36 years of age. At 46 years of age, insurance was refused because of a cardiac "condition." In September, 1932 he first observed dyspnea. On April 28, 1933 the electrocardiogram showed normal sinus rhythm with right intraventricular heart block. On July 31, 1933, the electrocardiogram showed 3 1 heart block. In December 1933 complete heart block occurred and persisted. He experienced Stokes Adams attacks occasionally The heart was enlarged. He exhibited no signs of congestive heart failure at the time these observations were made. The diagnoses were *A* arteriosclerosis *B*, cardiac en

TABLE II *

Data on patients exhibiting heart block and other rhythms associated with slow ventricular rate (Figure 4)

Case Hospital number Age	Date	Body surface	Oxygen consumption	Basal metabolic rate	Arterio venous oxygen difference	Cardiac output	Cardiac output	Heart rate	Cardiac output	Cardiac area	Cardiac volume	Arterial pressure	Left ventricular work	Circulation time‡	Venous pressure	Vital capacity	Rhythm	Red blood count	Hemoglobin
		sq m	cc. per minute	per cent	cc.	liters per minute	liters per sq m per minute	per minute	cc per beat	sq cm	cc	mm Hg	gram meters per beat	seconds	cm saline	cc.		millions	per cent
COMPLETE HEART BLOCK																			
H B No 26415 ♂ 56 years	Mar 23 1934	1 75	204	−10	79.3	2 58	1 47	35	73 7	134 9	1430	128/60	94.2			2710	C –H.B	5 0	104
	Mar 25, 1934	1 75	209	−8	76 0	2 75	1.57	35	83.3	134 9	1430	120/58	100 9			2670	C –H.B		
G N No 46694 ♀ 52 years	April 24 1934	1.53	148	−20	83 7	1.81	1 18	44	41 1	118.6	1186	120/80	55 9			1620	C –H.B	5 1	101
	May 1, 1934	1 53	149	−20	71 0	2 09	1.37	40	52.2	118 2	1163	108/68	62 5			1750	C –H.B		
A. R. No 4025 ♂ 49 years	May 16 1935	1 04	224	+4	67.3	3.33	2 03	37	90 0	145.2	1587	160/58	133 4	12 4	0 5	3600	C –H.B		
C K. No 150625 ♂ 54 years	Jan. 10, 1938	1 74	197	−11	64 8	3 04	1 75	32	95 0	141 6	1540	155/95	161 5	25 8 s 35 6 d	2.5	3650	C –H.B	4 7	98
2 1 HEART BLOCK																			
T C No 111203 ♀ 73 years	Nov 19 1935	1.60	159	−12	95.2	1 67	1 04	30	55 7	102.2	2428	150/68	82.6	32.2	5 8	2250	2 1 H.B	5 1	100
J L. No 41361 ♂ 71 years	April 27, 1936	1.87	228	+2	83.3	2 74	1 47	48	57 1	137.2	1466	164/80	94 7	23 1	5 7	3200	2 1 H.B	3 9	70
SINUS BRADYCARDIA																			
H J No 42048 ♂ 41 years	April 26, 1934	1.50	245	0	77 4	3 16	1 76	47	67.3	147.2	1620	118/86	93 4			3810	N.R.	5.8	102
COUPLED RHYTHM DUE TO AURICULAR PREMATURE CONTRACTIONS																			
J G † No 66753 ♀ 68 years	June 6 1934	2 00	218	−5	73.2	2 03	1 40	30	99 0	185 0	2205	190/140	217 6			2750	Idiovent. and A P C giving coupled rhythm	5.5	95
	June 9 1934	1 99	224	0	82.8	2 71	1.37	27	100 0	190 1	2304	190/130	222.2			3125			

* See Table I for abbreviations

‡ "s" and "d" indicate arm to tongue circulation time recorded during systole and the prolonged diastolic period respectively

† Figure 4 was not extended to include this patient

largement, *C*, complete heart block, right intraventricular heart block. Observations were made on March 23, 1934, as well as 2 days later (Table II)

G N (N Y H No 46694), a white female, aged 52 years, suffered from complete heart block which had its onset during an attack of diphtheria at 9 years of age. She was admitted to the hospital on April 21, 1934. At 12 years of age, she suffered an attack of acute rheumatic fever with recurrences at 24 and 35 years of age. She suffered cardiac symptoms first in November 1933. There was precordial pain accompanied by palpitation and dyspnea and constriction of the chest, lasting one to two minutes. These attacks increased to 6 to 8 every day but after digitalization in the outpatient department the at-

tacks decreased to one or two. After resting two weeks, the attacks increased again in frequency when she attempted greater activity. She was admitted to the hospital April 21, 1934, to be given larger amounts of digitalis. The diagnoses were *A*, diphtheria, *B*, cardiac enlargement, *C*, complete heart block. She received digitalis, 1 0 gram, on April 22 and 23, 1934. At rest there were no signs of congestive heart failure. Studies of the circulation were made on April 24, 1934 (Table II) and were repeated on May 1, 1934. She had received digitalis, 0 2 gram, daily except on April 26th when the amount was 0 7 gram.

A R (N Y H No 4025), was a white male aged 49 years. At 26 years of age, a routine physical examina-

tion showed a "slow pulse and a heart murmur" Life insurance was refused at 31 years because of these At 41 years he became easily fatigued. He suffered an at tack of rheumatic fever at 42 years followed by a second attack at 45 years The electrocardiogram showed in complete auriculoventricular heart block in February, 1932, complete heart block had been present, however since September, 1932. At the time our observations were made, he experienced slight precordial pain, and dyspnea on exertion. Examination revealed no signs of congestive heart failure. The diagnoses were *A*, rheu matic fever, hypertension *B* mitral insufficiency *C* complete heart block. On May 16 1935, studies of the circulation were made (Table II)

C K (N Y H No 189625) a white male, aged 54 years was admitted to the hospital on December 31, 1937 complaining of dizzy spells' He had never suffered from rheumatic fever nor chorea He had enjoyed ex cellent health until 10 weeks before admission when he began to experience cramps in the calves of both legs on walking Eight weeks before admission he began to suffer from moderate dyspnea on exertion and five weeks before admission he experienced an attack characterized by the successive appearance of whistling in the ears whirling vertigo and brief loss of consciousness The entire succession of events lasted about 60 seconds At tacks of this nature recurred daily in the 3 weeks pre ceding admission. Examination on December 31, 1937, revealed no evidence of heart failure of the congestive type. Observation of the venous pulsations in the neck together with auscultation at the apex of the heart indi cated that the rhythm was complete heart block with a ventricular rate of 30 per minute. The peripheral ar teries were moderately thickened and tortuous but the arterial pulses at both wrists appeared to be of good volume. The diagnoses were *A* arteriosclerosis *B* enlargement of the heart, fibrosis of the myocardium *C*, complete heart block alternating with incomplete heart block, right intraventricular heart block. The patient was kept at rest in bed, and did not experience any fur ther attacks. On January 6 1938 he was given ephedrine sulphate 0 025 gram, 4 times daily The drug did not appear to induce any significant change in the ventricular rate. Studies of the circulation were made on January 10 1938 when complete heart block was present.

Summary of data relating to complete heart block

Studies were made of 4 patients exhibiting com plete heart block without congestive heart failure The cardiac output per minute was decreased in three and normal in one, and the output per beat was increased or only slightly decreased The basal metabolic rate was decreased in three sub jects

There are studies of 2 patients exhibiting 2 1 heart block (T C and J L., Table II)

T C (N Y H No 111203), a white female, aged 73 years, was admitted to the hospital on October 30, 1935, and discharged November 24 1935 She exhibited signs of congestive heart failure (ascites dyspnea, slight edema) beginning 16 months before admission. The heart was enlarged. There was marked arteriosclerosis. The electrocardiogram showed auricular flutter (auricu lar rate 272 per minute) with complete heart block ven tricular rate 30 per minute. On November 19 1935 how ever normal sinus rhythm was present, with 2 1 heart block the ventricular rate was 30 per minute. The pa tient now being free of signs of congestive heart failure, the cardiac output was measured (Table II) The diag noses were *A* hypertension *B*, cardiac enlargement *C* 2 1 heart block right intraventricular heart block.

J L. (N Y H No 41361) a white male, aged 71 years, was admitted to the hospital on April 23 1936 because of symptoms of early prostatic obstruction. Hypertension had been present for many years and the patient had suffered right hemiplegia 9 years before. Heart block, 2 1 was discovered in 1934 The radial vessels were thickened. At rest there was no evidence of congestive heart failure. The diagnoses were *A* hypertension *B* enlarged heart *C* 2 1 heart block Studies of the circulation were made on April 27 1936 (Table II)

Summary of data relating to 2 1 heart block

The total cardiac output per minute was de creased in the presence of 2 1 heart block as a consequence of which the circulation time was prolonged. The output per beat, due to the slow cardiac rate, was within normal limits

H J (N Y H No 42048) a white male, aged 41 years was admitted to the hospital on April 13, 1934, suffering from dry pleurisy of 10 days duration. There was no rise in temperature. Examination of the sputum did not reveal acid fast organisms The friction rub and pleural pain disappeared within a few days and con valescence was uneventful. The x ray photograph of the chest revealed no evidence of tuberculosis There was no history of rheumatic infection. Examination of the heart and circulation revealed no abnormality Sinus bradycardia was present. There were no signs of con gestive heart failure. On April 26 1934, when the heart rate was 47 per minute, the cardiac output was measured (Table II)

Coupled rhythm due to auricular premature contractions

J G (N Y H No 66753) a white male aged 68 years was admitted to the hospital on June 1 1934 be cause of the presence of dyspnea and moderate edema of several weeks duration. He had been given small amounts of digitalis for 5 days but none had been given

Our own observations relating to congestive heart failure give evidence which points to the same conclusion (19, 24)   The graphic representation of patients exhibiting complete heart block in the zone of normal circulatory function is in agreement with the clinical observation that patients suffering from this rhythm carry on for long periods without experiencing congestive heart failure   The decrease in cardiac output which was present in 4 of our cases and 2 of Starr's (8) calls for comment since other observers have reported normal values (Grollman (11), p 221) The cases we are now reporting were in the middle decades, yet the range from decrease to normal was recorded   We are unable to express an opinion whether arteriosclerotic changes may account for the difference in functional capacity It is recalled again, however, that all fell into the zone of normal circulatory function (Figure 4)

Our data show that the basal metabolic rate may be low in the presence of complete heart block and 2 1 heart block, a phenomenon to which others have already directed attention (8)   This is probably a compensatory mechanism on the part of the human organism, for, by decreasing the total oxygen requirements of the tissues, the cardiac output, though diminished, may be utilized to its fullest extent   This compensatory mechanism is equivalent to presenting this smaller minute volume output to an individual with a smaller body surface, for whose requirements it would be ample

The two patients exhibiting sinus bradycardia and coupled rhythm (H J and J G, Table II, Figure 4) require no special comment, since the situation in them is essentially the same as in those having heart block

SUMMARY

Auricular fibrillation, auricular flutter, and paroxysmal tachycardia, of the supraventricular as well as the ventricular type were associated in the instances observed with decreased cardiac output per minute, per beat, decrease in velocity of blood flow, and dilation of the heart, and decrease in the work of the heart per beat   As a consequence, these patients fell in the heart failure zone when the abnormal rhythm was present The cardiac output per minute was likewise usually decreased in heart block, but, in contrast to
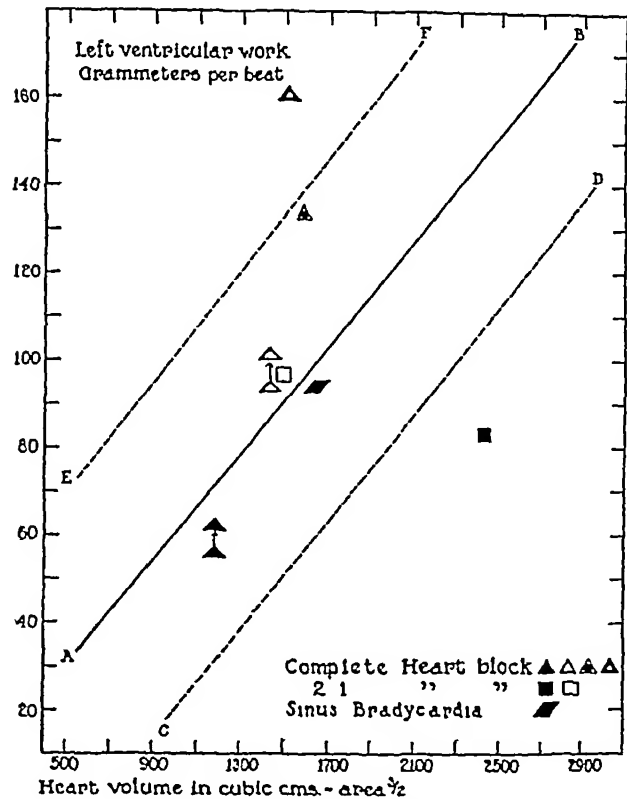
FIG 4   LEFT VENTRICULAR WORK PER BEAT AND CARDIAC VOLUME

The data from Table II relating to work of the left ventricle per beat in the rhythms associated with a slow ventricular rate are plotted against the corresponding cardiac volumes, in a manner similar to Figure 3   The open and the closed triangles, the triangle with a dot, the triangle within a triangle, the open and the closed squares, and the closed parallelogram refer respectively to the cases in the sequence they are recorded in Table II   All patients fell in the zone of normal circulatory function except T C. (solid square) who had recently recovered from congestive heart failure.

the rapid rhythms, the output per beat was increased, and the work per beat commensurate with the size of the heart   The work of the heart was normal except in the instance in which the patient had recently suffered congestive heart failure   A patient exhibiting sinus bradycardia, as well as one with coupled rhythm, at rest, resembled those with heart block and fell in the zone of normal circulatory function

CONCLUSION

The rapid regular and irregular rhythms in human beings at rest are associated with marked decrease in functional capacity of the heart, as measured by cardiac output per minute and per beat

and the work per beat. They were associated with the dilatation of the heart. They are very inefficient rhythms, the work of the left ventricle per beat not being commensurate with the size of the heart. As a consequence, in most instances they fall outside the zone of normal circulatory function. On the other hand, rhythms associated with slow ventricular rate such as those illustrated by complete heart block, are not incompatible with a normal circulatory function when the subject is at rest. Patients suffering from these rhythms may exhibit lowering of the basal metabolic rate as a compensatory mechanism.

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TABLE I

Subject 1　　Normal male, age 23 years, weight 73 kilograms

Date	Time	Medication	Urine			Blood			
			Volume	CO_2 content	pH	Serum CO_2 content	Serum pH	Sulfanilamide concentration	Methemoglobin concentration
1937			*cc*	*volumes per cent*		*volumes per cent*		*mgm per cent*	*per cent**
October　30	9·00 a m		126	4 1	5 29				
	9.55		90	6 8	5 63				
	11 00		272	18 5	6 14				
	1.30 p m		270	30 5	6 74				
	4 15		181	18 1	6 27				
	6.30		221	17 9	6 22				
November 1	8.55 a m					73 0	7 40		
	9 00	14 7 grams sulfanilamide from 9·00 to 11·00 a m	44	3 3	4 97				
	9.50		43	18 2	6 20				
	10.50		150	150 0	7 37				
	11 45		170	170 0	7 55				
	1 26 p m		446	179 0	7 53				
	1.35					66 5	7 43	23 8	13
	3.30		398	161 0	7 51				
	4.30					66 6	7 40		13
	4.35		199	148 0	7 55				
	6.30		326	123 0	7 43				
	9.35		275	109 0	7 35				
November 2	9·05 a m		329	45 0	6 76				
	11·00							9 2	10

* Methemoglobin concentration expressed as per cent of total hemoglobin pigment

tion of bicarbonate, there was a fall in the serum carbon dioxide content which ranged from 5 5 to 24 7 volumes per cent　During this period, we observed in every case a slight rise in the serum pH

The production of alkaline urine with simultaneous reduction of the carbon dioxide content of the blood serum and rise in serum pH has been described by a number of investigators (10, 11, 12) as the result of voluntary hyperventilation The accepted explanation for these changes is as follows　The hyperventilation lowers the carbon dioxide tension of the alveolar air and consequently that of the plasma　The immediate result of the decreased plasma carbon dioxide tension is an increase in the ratio of $\dfrac{BHCO_3}{H_2CO_3}$ with an increase in the plasma pH　One manifest compensatory response to this alteration is the increased excretion of bicarbonate by the kidney in an effort to reestablish the normal ratio between bicarbonate and carbonic acid, upon which the pH of the plasma depends　The reduction of

base bicarbonate in the serum probably should not be attributed wholly to the excretion of bicarbonate by the kidney　At an elevated pH of the blood serum, other blood buffers, particularly phosphates and proteinates, claim more base, which is yielded by bicarbonate　In addition, chloride shift from the red blood cell to the plasma would tend further to reduce bicarbonate, as would also the slight and transient ketosis which seems frequently to be associated with the alkalosis of hyperventilation (13), and which we have also noted following sulfanilamide administration Aside from such mild ketosis, however, there seems to be no other acid accumulation, certainly there is no significant rise in lactic acid, which conceivably might occur as a result of anoxemia secondary to methemoglobin formation　Accumulation of phosphate has been shown not to occur and elevation of serum sulphate seems very unlikely　Even if abnormal acid accumulation were to occur and explain the reduction in serum bicarbonate, we would still have to explain the rising pH of the serum

Our findings in regard to the direction of the

TABLE II
Subject 2   Normal female, age 33 years   weight 47 kilograms

Date	Time	Medication	Urine			Blood				
			Volume	CO₂ content	pH	Serum CO₂ content	Serum pH	Sul fanil amide concen tration	Met hemo globin concen tration	Serum lactic acid
1937			*cc.*	*volumes per cent*		*volumes per cent*		*mgm per cent*	*per cent*	*mgm per cent*
November 2	9.53 a m		160	44 7	6 82					
	10.55		120	27.2	6 82					
	11.53		220	12 7	6 43					
	1.26 p m		229	5.3	5 85					
	3·00		121	22.5	6 65					
	4·45		179	15 1	6 43					
November 3	9 15 a m.					67 2	7.37			
	9.26		81	6 5	5 84					
	10.27	9 4 grams sulfanila amide from 9.27 to 11.27 a m.	186	96 5	7.35					
	11:05		200	89 7	7.39					
	11.57					54 2	7 42		5	
	12·02		291	86.3	7 44					
	1 11 p m		198	121 0	7.51					
	2.30		53	98 7	7.57					
	3·00		251	91.3	7 48					
	3·45		86	127 0	7 73					
	4.30					50 5	7 43		8	23 2
	5 15		246	163 0	7 76					
	6.50		84							
	8·45		108	Alkaline to bromcresol purple						
	10.30		182							
November 4	2:00 am		153							
	6:00		250							
	7.57		142	5.3	5 60					
	9 15					42 5	7 40	4 7		
	1·45 p m.					50 8	7.38			

pH change of the blood serum are in apparent disagreement with those reported by Marshall *et al* (5) in dogs   It can be seen from our data that the pH values for the blood serum increased in every experiment.   Although the changes were not very marked in some of the experiments, they become more significant when we consider that if the decrease in the carbon dioxide content of the blood serum were to be explained on the basis of acidosis, one would expect a definite fall in the pH value of the serum of about 0 10 (14) In the six experiments on three dogs reported by Marshall, the first actually showed a rise in serum pH of 0 06 during the period in which the carbon dioxide content of the serum fell 17 5 volumes per cent   In the second, and the only one in which an abnormally low pH was obtained, the carbon dioxide content of the serum actually rose during the period in which the pH of the serum was shown to fall.   In the third, the variation in

the pH of the serum during the control period was as great as the interpreted fall during the test period, and the pH of the serum at the end of the experiment was the same as at the beginning In the other three experiments, a fall in the pH of the serum was obtained simultaneously with the reduction in the carbon dioxide content of the serum, but in only two of these was the fall commensurate with that expected from the degree of reduction of the carbon dioxide content of the serum (14)   Whether, as Marshall suggests, some of his apparently discrepant results are owing to respiratory disturbances because of difficulty in controlling the experimental animals, and whether the difference between his findings and ours depends upon the larger doses of sulfanilamide which he used, cannot be stated, but it seems justifiable to conclude from our data obtained on human subjects that the change in the acid base balance is that of a carbon dioxide deficit type of

TABLE III

Patient 1   Female, age 14 years, weight 55 kilograms   (Subacute bacterial endocarditis)

Date	Time	Medication	Urine			Blood				
			Volume	CO₂ content	pH	Serum CO₂ content	Serum pH	Sulfanilamide concentration	Methemoglobin concentration	Serum lactic acid
1937			*cc*	*volumes per cent*		*volumes per cent*		*mgm per cent*	*per cent*	*mgm per cent*
October 23	11 00 a m			3 9	5 22					
	7.30 p m			6 2	5 78					
October 24	2·00 pm			5 7	5 66					
October 25	9 20 a m	11 0 grams sulfanilamide from 8 45 a m to 12·00 m		3 4	4 85					
	9.30					67 3	7 37			
	12·00 m		242	161 0	7 69					
	1 25 p m		122	104 0	7 60					
	2 15					61 0	7 42	21 7	12	
	4·00	11 0 grams of sulfanilamide per 24 hours in 6 doses	268	197 0	7 79					
	7 15		132	207 0	7 87					
	8·00					56 0	7 45	17 6	10	
	10·00		122	168 0	7 69					
October 26	3.50 a m		116	13 8	6 13					
	9·00					50 0	7 40	17 9	11	
	9·05		139	18 8	6 42					
	12.35 p m		134	49 8	6 99					
	3 40		126	73 0	7 12					
	5 10		46	36 3	6 89					
	8 15		100	39 6	6 74					
October 27	7.50 am		296	5 2	5 08					
	8·00					51 0	7 37		6	23 9

alkalosis rather than that of an alkali deficit type of acidosis

Disregarding the discrepancies in serum pH data, our conclusions still seem logical   The only other explanation for a fall of serum carbon dioxide content with a simultaneous increase in urinary pH to values above 7 is, as Marshall suggests, a failure of the tubules to reabsorb bicarbonate, and there is considerable evidence against this supposition   In several of our experiments the urines were tested for dextrose, and none of them produced any reduction of Benedict's qualitative reagent, so that dextrose, at least, was reabsorbed normally   Aside from alkalosis, the only commonly known cause for failure of reabsorption of bicarbonate by tubular epithelium is the very rapid passage of urine through the tubules leading to polyuria, which was true neither in Marshall's experiments nor to an extensive degree in our own   Marshall, on the contrary, has shown that the rate of glomerular filtration is decreased and that there is no pathological or laboratory evidence of kidney damage with the doses of sulfanilamide employed   Besides these indirect evidences against the possibility of failure of the tubules to reabsorb bicarbonate, an experiment to be described later in this paper offers direct evidence that such is not the case   The data for this experiment are given in Table V   Ammonium chloride was given preliminary to the administration of sulfanilamide, and the urine became practically free of bicarbonate   It can be seen from Table V that the pH of the urine remained as low for five hours after the sulfanilamide was given as it was during the two hours prior to its administration   If there were to have been failure of the tubules to reabsorb bicarbonate due to the sulfanilamide, there is no reason to suppose that ammonium chloride would have prevented this effect   We believe, therefore, that the fall of serum carbon dioxide content following sulfanilamide administration may in most instances be explained primarily on the basis of carbon dioxide deficit resulting from hyperventilation

In some cases, hyperventilation may not be

TABLE IV

Patient 2   Female, age 9 years weight 20 kilograms   (Pyelitis)

Date	Time	Medication	Urine			Blood			
			Volume	CO₁ content	pH	Serum CO₂ content	Serum pH	Sulfanilamide concentration	Met hemoglobin concentration
1957			*cc*	*volumes per cent*		*volumes per cent*		*mgm per cent*	*per cent*
November 4	5·45 p m		216	24.5	6 20				
November 5	6 10 a m.		91	4 7	5 41				
	8·45	Methylene blue 2 cc. 1 per cent solution intravenously				58 7	7 42		
	9.20	4 0 grams, sulfanilamide from 9·00 to 11·00 a. m.	51	4 9	5 65				
	10.35		159	62 7	7.33				
	11 10		90	39 8	7.27				
	11.30	Methylene blue 1 cc. 1 per cent solution intravenously				61.5	7 43	11.2	Less than 3
	11·40		161	63 6	7.53				
	1.55 p m		126	127 0	7 70				
	3.35					53 2	7 45		Less than 3
	3·40		141	141 0	7 75				
	4:00	4 0 grams of sulfanilamide per 24 hours in 6 doses							
	7·00		98	149 0	7 75				
	8 10					53 0			
	8 15		60	112.0	7 65				
	9.50		90		7 20				
	11:00	Sulfanilamide stopped							
November 6	8:00 a.m.	Sulfanilamide started 2 0 grams per 24 hours							
	11·00		121	71 2	7.51				
	12 10 pm.		19	73 4	7 77				
	1·45		60	60.5	7 51				
	2:00					48.2	7 45	5 0	
	3:05			36.9	7 15				
	3.55				7.26				
	4.30				7 27				
November 7	10:00 a.m				6 87				
	10.25				7 17				
	12·45 p m.					48 0		4 1	

particularly noticeable, but in certain of our subjects it was quite marked   Two patients, one child and one adult, on whom we do not have preliminary data, showed very marked increase both in depth and rate of breathing after having received both large and continued doses of sulfanilamide.   At the time when the hyperventilation was extreme, the serum pH values were found to have reached 7 55 and 7 57, respectively In the child, signs of tetany were present.   The effectiveness of the hyperventilation in producing carbon dioxide deficit depends more upon increase in depth of respiration than increased rate, and therefore overbreathing may be easily overlooked until it becomes quite marked

In endeavoring to explain the hyperventilation,

we wished particularly to determine whether the sudden reduction in the oxygen carrying capacity of the blood resulting from the rapid accumulation of methemoglobin might be largely responsible.   We therefore prevented the accumulation of any appreciable amount of methemoglobin in Patient 2 by the injection of methylene blue (7) Despite the fact that the methemoglobinemia was extremely slight, the patient obviously hyperventilated and the usual chemical changes ensued Furthermore there appeared to be no correlation in other cases between the degree of methemoglobin accumulation and the extent of hyperventilation

Symptoms which developed following these large doses of sulfanilamide varied considerably

in different individuals, but those most consistently noted were as follows   Within thirty minutes after the first dose had been taken, vertigo was experienced, and within two hours, hyperventilation, nausea, anorexia, increased thirst, and cyanosis were also noted   These symptoms continued and were accompanied by drowsiness, irritability, and very evident mental confusion involving principally false perception of intervals of time, extreme difficulty in concentration, and slow response to simple questions   Usually there was no gross disorientation   Recovery from the subjective symptoms, in the normal subjects, was not complete for forty-eight hours, with vertigo, anorexia, and drowsiness persisting throughout the day following drug administration   It is interesting to note the similarity of many of these symptoms to those described by Collip and Backus (10) and Lepper and Martland (12) in subjects who voluntarily overbreathed and became alkalotic   Dizziness, drowsiness, and increased thirst were characteristic, as well as irritability, unreasonableness, and a lowering of the critical faculty

To test to what extent the subjective symptoms which we observed following the administration

TABLE V

Subject 2   Normal female, age 33 years, weight 47 kilograms

Date	Time	Medication	Urine			Blood					Scott Wilson expired air*
			Volume	pH	Ferric chloride*	Serum CO₂ content	Serum pH	Serum inorganic phosphorus	Serum lactic acid	Sulfanilamide concentration	
1937			*cc*			*volumes per cent*		*mgm per cent*	*mgm per cent*	*mgm per cent*	
December 26	11 15 p m					61 2	7 42	3 8			
December 27	12·05 a m	Ammonium chloride 2 grams									
	3 00	Ammonium chloride 2 grams									
	6·00	Ammonium chloride 2 grams									
	7·05–8 00		77	4 66							
	8.30–9.37		167	4 68	0						
	10 14					50 5	7 35			15 8	
	10 15	Ammonium chloride 3 grams 9 7 grams sulfanilamide from 10 15 a m to 12 15 p m									
	10 23		92	4 48	0						
	11 13		248	4 57	+						
	11 15	Ammonium chloride 3 grams									
	11 45										+
	12 15 p m		238	4 44	+						
	1 03		275	4 45							
	1 15					37 3	7 32	3 4	14 5	11 2	
	1.30		217	4 57	+						
	3·00		350	4 47	+						
	4 15					35 0	7 30				
	4 50		163	4 82	+						
	7 18					37 6			18 5	9 4	
	7 20		216	4 85	+						+
December 28				Acid to methyl red							
	8.50 a m		56	5 02	+						

* It will be noted from the data in Table V that within an hour after the first dose of sulfanilamide, the urine gave a slightly positive test with ferric chloride and the reaction became strongly positive within four hours, and remained so for two days   The color which developed with ferric chloride was not the typical Bordeaux red customarily seen with clinical ketosis, but was more purple and very intense   The Rothera test also was positive   Coincident with the positive ferric chloride tests in the urines, positive Scott-Wilson tests were obtained when the subject blew through this reagent   When the serum filtrates were distilled into Scott-Wilson reagent preliminary to lactic acid determinations, positive tests also resulted   Quantitative estimations of acetone bodies were not made at the time of the experiment, but some days later one of the specimens of urine which still showed strongly positive ferric chloride and Rothera tests, when refluxed with Van Slyke's mercuric sulfate reagent (15), gave insignificant amounts of precipitate   Further investigations of these findings are to be made in an effort to determine the significance and the extent of the ketosis which is suggested by the positive qualitative tests, and to determine the rôle that ammonium chloride may have had in producing these reactions, which persisted for an unusually long period

of the drug are related to alkalosis and to what degree tolerance may be acquired by repeated administration the following experiments were made Over a period of twelve hours, one of the normal adults, Subject 2, was given the amount of ammonium chloride calculated to lower the carbon dioxide content of the body fluid 15 volumes per cent, and, at the end of this period, was again given 0 2 gram of sulfanilamide per kilo gram in two hours All of the former determinations were repeated, the results of which are given in Table V From this data it can be seen that the development of alkalosis was prevented, the pH of the urine remained consistently below 5 for twenty three hours, and the pH of the blood serum fell from 7 42 to 7 30 Particular attention was given to comparing the subjective symptoms experienced during this experiment with those which developed during the former one on this subject In general, all the previously described symptoms were less marked except the hyperventilation which subjectively seemed more severe. However, when the sulfanilamide experiment on Subject 1 was repeated, the results of which are given in Table VI, and in which the alkalosis was again allowed to develop, comparable diminution in the symptoms likewise was noted

We feel, although the drug was not taken a third time by Subject 2, that the decreased intensity of the symptoms may be explained possibly on the basis of an acquired tolerance to the drug rather than to prevention of alkalosis

Even if it is later definitely found that alkalosis may not play an important role in the production of disagreeable symptoms, routine alkali administration in conjunction with sulfanilamide as recommended by Long and Bliss and adopted by others seems to us not only not indicated but definitely undesirable under certain circumstances It is true that amounts of alkali as recommended (10 grains of sodium bicarbonate with each dose of sulfanilamide) and even much more in the form of sodium lactate may at times be rapidly excreted into the urine without interfering with compensation for carbon dioxide deficit (4) When however, a deficiency of total fixed base in the body fluids is present (such as occurs for instance when marked vomiting with loss of acid gastric juice is associated with acute infections of the urinary tract), the physiological renal response is restriction of base excretion into the urine and the consequent formation of acid urine (low in bicarbonate) In such cases another type of alkalosis is often present—due to excess of

TABLE VI

Subject 1   Normal male age 23 years, weight 73 kilograms

Date	Time	Medication	Urine		Blood				
			Volume	pH	Serum CO₂ content	Serum pH	Sul fanil amide concen tration	Met hemo globin concen tration	Serum inor ganic phos phorus
1937			*cc*		*volumes per cent*		*mgm per cent*	*per cent*	*mgm per cent*
December 29	9-05 a. m.			5 44					
	10-05				75 0	7.35			3 8
	10.23		123	6.35					
	10.30	15 0 grams sulfanilamide from 10.30 a.m to 12.30 p m							
	11.23		162	7 24					
	12:20 p m.		544	7 47					
	1 15		367	7 45					
	1-45				67 9	7 40	18 0	13	4 0
	1.55		340	7.35					
	3-00		322	7.55					
	4.30	Methylene blue 7.5 cc. 1 per cent solution intravenously			63 8	7 40	18 9	16	
	5.20		392	7 67					
	7-45		203	7 59					
	8-00				65 0		15 4		
	9.20	Alkaline to bromcresol purple							
	10.50								
December 30	10.50 a.m								

bicarbonate in the blood, compensation for which is depressed respiration and not excretion of alkali into the urine Further alkali administration could only increase the degree of alkalosis, and add to its danger

It should be noted from the tables, however, that the reaction of the urine may swing back to the acid zone, even though sulfanilamide administration is continued The implications of the explanation of this are of interest When the reduction of the base bicarbonate in the serum has reached the level at which, despite the decrease in carbonic acid brought about by hyperventilation, the ratio of bicarbonate carbonic acid has again reached the normal value of 20 1, then there is no further need to excrete bicarbonate into the urine, and the latter may again become normally acid At this time, the pH of the blood should be lower than it was when bicarbonate was being excreted in excessive amounts, and the data confirm this expectation The carbon dioxide content of the serum at this same time would still be reduced because both bicarbonate and carbonic acid are below normal levels It is easily conceivable that, with this existing bicarbonate deficit, a true acidosis might readily develop should ketosis, lactic, or other acid accumulation occur, or, on the other hand, should the hyperventilation suddenly cease, allowing carbonic acid to accumulate This also has a bearing on the treatment of urinary tract infections If it is desired to keep the urine alkaline in such subjects, this could be accomplished more safely by administration of sodium lactate from which alkali becomes available gradually It should be remembered, however, that chloride, if deficient, may also have to be administered in order for alkali to be excreted into the urine, and for alkalosis of the base bicarbonate excess type to be avoided (16) Simultaneous administration of both substances may be accomplished very satisfactorily by using mixtures of molar sodium r-lactate and Ringer's solution When mixed in equal parts, one to two cc per kilogram given every four hours should maintain alkalinity of the urine with safety, provided extreme degrees of renal insufficiency are not present The substitution of sodium lactate for sodium bicarbonate also has the advantage of preventing undesirable reduction of gastric acidity

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AN "ACID" PHOSPHATASE OCCURRING IN THE SERUM OF PATIENTS WITH METASTASIZING CARCINOMA OF THE PROSTATE GLAND

By ALEXANDER B GUTMAN AND ETHEL BENEDICT GUTMAN

(From the Department of Medicine College of Physicians and Surgeons Columbia University and the Presbyterian Hospital New York City)

In 1935, Kutscher and Wolbergs (1) found that normal prostate tissue is extraordinarily rich in a phosphatase with optimum activity at about pH 5 0 This observation was confirmed for normal and carcinomatous prostate tissue by Gutman, Sproul, and Gutman (2), who further noted the presence of "acid" phosphatase at the site of skeletal metastases secondary to carcinoma of the prostate gland

The present investigation was directed toward the possibility that invasion of lymph or blood channels by prostate carcinoma might result in the escape into the circulating fluids of prostate phosphatase sufficient to cause a measurable increase in the "acid" phosphatase activity of blood *serum* Significant amounts of such an "acid" phosphatase were found in the serum of 11 of 15 patients with disseminated carcinoma of the prostate gland The "acid" phosphatase noted in the serum of these patients corresponds closely in its characteristics with prostate tissue phosphatase, as described by Kutscher and Wörner (3) Its properties differ in significant respects from those of recognized phosphatases of the blood

With the exception of one case, no appreciable rise in "acid" serum phosphatase activity was noted in a variety of diseases other than carcinoma of the prostate gland, including conditions presenting marked increases in "alkaline" serum phosphatase activity The determination proved to be helpful in the diagnosis of disseminated carcinoma of the prostate gland

METHODS

The method of King and Armstrong (4) has been shown to be readily adaptable to the estimation of phosphatase activity on the acid side of neutrality (2) Preliminary experiments indicated that pH 4.9 was within the optimum range of activity of the serum phosphatase with which we are here concerned and beyond the range of

significant activity of "alkaline" serum phosphatase. The buffer substrate employed was M/200 disodium monophenylphosphate in Sørensen's M/10 citrate—HCl buffer adjusted to pH 4 8 After addition of serum, the pH of the reaction mixture at room temperature was 4 85 (glass electrode measurements), and therefore approximately 4.9 at the temperature of hydrolysis, 37° C The time of hydrolysis was 3 to 5 hours, except in most cases of metastatic prostate carcinoma in which the optimum time of hydrolysis was ½ to 1 hour Apart from these deviations, the procedure followed was essentially that outlined by King and Armstrong (4) The results are expressed in units of phosphatase activity per 100 cc. of serum. A unit is defined as that degree of phosphatase activity which at pH 4.9 and 37° C will liberate from the specified buffer monophenylphosphate substrate solution 1 mgm of phenol in 1 hour

"Alkaline" phosphatase activity of the serum was determined by the method of Bodansky (5)

RESULTS AND DISCUSSION

"Acid" phosphatase activity of normal serum Normal sera consistently show slight but measurable hydrolysis of monophenylphosphate substrate buffered with citrate—HCl to pH 4.9 (6, *cf* Roche (7)) The range of variation in normal subjects tentatively appears to be 0.5 to 2 5 units (6), as defined above.

That the hydrolysis is enzymatic is indicated by the absence of scission in control experiments in which substrate-buffer mixtures were maintained at 37° for 5 hours without the addition of serum A study of this reaction (6), suggests that the enzyme involved is not "alkaline" serum phosphatase. Normal serum apparently contains minute amounts of one or more phosphatases of the type classified by Folley and Kay (8, 9) as phosphomonoesterase A, The properties of this "phosphatase do not coincide with those of

No significant rise in " acid " serum phosphatase activity was found in diseases of the prostate gland other than carcinoma with metastases (Table IB)

Comparison of properties of prostate tissue phosphatase with those of " acid " phosphatase of the serum of patients with disseminated carcinoma of the prostate gland The results summarized in Table II show satisfactory agreement between the pH-activity curves of prostate tissue phosphatase and those of serum phosphatase in two cases of carcinoma of the prostate gland with metastases The dilutions indicated in the table compensate for differences in concentration of the enzyme in prostate tissue and in serum In order to approximate initial reaction velocities, the time of hydrolysis was made as short as was consistent with accurate readings Comparison with the data of Kutscher and Worner (3) shows a general correspondence in the broad range of optimum activity but with the concentration of substrate and conditions of hydrolysis selected, our peak of activity was found to be closer to neutrality

In confirmation of Kutscher and Worner (3), the activity of prostate tissue phosphatase was found to vary widely with different concentrations of β glycerophosphate substrate (Table III) This effect was less pronounced when molarities of disodium monophenylphosphate substrate varying within the range investigated (Table III) were employed at the same pH Similar results

were obtained with serum from a case of disseminated prostate carcinoma (Table III)

Prostate tissue phosphatase is unusual in that it is rapidly and irreversibly inactivated by alcohols (3) The " acid " phosphatase activity of the serum in patients with carcinoma of the prostate gland is likewise inhibited by alcohols A 1 20 dilution of the serum of Case 1, with 24 9 units of phosphatase activity, yielded a value of 17 6 units after one hour's incubation with added N-propyl alcohol in a concentration of M/1 in the reaction mixture The hydrolysis in both experiments was conducted at 37° C with M/200 monophenylphosphate substrate in M/10 citrate buffer at pH 5 06

A parallel experiment performed under the same conditions, except for the use of sodium fluoride in a concentration of M/100 in the reaction mixture, resulted in reducing the activity of this serum (Case 1) from 24 9 to 5 6 units As regards the inhibiting effect of fluoride too, then, the " acid " serum phosphatase corresponds with prostate tissue phosphatase The " alkaline " phosphatase of serum, on the other hand, is not significantly inhibited by fluoride (8) This fact was utilized to contrast the effect of fluoride on the phosphatase activity of the serum in a patient with disseminated prostate carcinoma who presented elevated levels of both " acid " and " alkaline " phosphatase activity (Case 3) At pH *9 0*, a sample of this serum showed no decrease in activity when hydrolysis was carried out for one-

TABLE III

Effect of variations in substrate concentration on activity of prostate tissue phosphatase and on activity of serum phosphatase from a case of metastasizing prostate carcinoma

A SUBSTRATE = β GLYCEROPHOSPHATE
(Buffer M/25 citrate, time 1 hour, pH 4 37, t = 37° C)

Molar concentration of substrate	0 006	0 0125	0.017	0 025	0 034	0 05	0 10	0 15	0.20	0.30
Normal prostate tissue (*mgm phenol liberated per 100 cc extract (1 gram of wet tissue 2360 cc of water)*)	4 0	5 4		11 5		13 7	14 9	14 8	12 4	9 5
Serum of Case 1 (*mgm phenol liberated per 100 cc serum (1 20 dilution)*)	3 5	5 3	8 6	10 3	11 3	12 5	13 7	13 5	11 6	8 0

B SUBSTRATE = MONOPHENYLPHOSPHATE
(Buffer M/10 citrate, time 1 hour, pH 4 37, t = 37° C)

Molar concentration of substrate	0 0025	0 005	0 01	0 02	0 05
Normal prostate tissue (*mgm phenol liberated per 100 cc of extract (1 gram of wet tissue 2360 cc of water)*)	19 8	20 9	23 7	23 5	26 9
Serum of Case 1 (*mgm phenol liberated per 100 cc of serum (1 20 dilution)*)	18 7	19 4	20 0	22 2	26 0

half hour at 37° C. with sodium fluoride of M/20 concentration in the reaction mixture. The same serum liberated 18.3 mgm. of phenol at pH 4 4 but, at this pH, the conditions of hydrolysis otherwise unchanged, sodium fluoride of M/20 concentration in the reaction mixture reduced the activity in one half hour to 2.2 mgm of phenol liberated per 100 cc serum

A sample of the serum of Case 3 was employed also for a similar experiment with magnesium salts, which are known to activate "alkaline" serum phosphatase but have no effect upon prostate tissue phosphatase (3) At pH 9 0, under the conditions of hydrolysis outlined in the preceding paragraph, 50 2 mgm phenol were liberated in the presence of magnesium chloride (M/50 concentration in the reaction mixture), as contrasted with 33 0 mgm phenol without the addition of Mg ion. At pH 4 4, conditions of hydrolysis otherwise unchanged, 18.3 mgm. of phenol were liberated both in the presence and in the absence of added M/50 magnesium chloride. In another instance (Case 9), hydrolysis of M/200 phenylphosphate for 2 hours at pH 5.97 and 37° C with M/20 magnesium chloride added yielded a value of 15 7 units as compared with 15 8 units without Mg ion

The absence of any activating effect of magnesium salts on the ' acid ' phosphatase activity of the serum in patients with metastasizing prostate carcinoma contrasts with the marked activation of the "acid" phosphatase found in normal erythrocytes (7) These two "acid" phosphatases differ also with respect to their capacity to hydrolyze α glycerophosphate. Unlike erythrocyte phosphatase, the "acid ' phosphatase found in the serum of patients with disseminated prostate carcinoma hydrolyzes the α isomer less rapidly than β glycerophosphate. A sample of the serum of Case 1 in 1 5 dilution liberated 172 mgm of phosphorus per 100 cc. in one hour from a β glycerophosphate substrate at pH 4.9, but only 87 mgm of phosphorus from an α glycerophosphate substrate of the same molarity and under the same conditions of hydrolysis [1] Kutscher and Wolbergs (1) report that prostate tissue phosphatase splits about 10 per cent less of α glycerophosphate than of the β isomer

[1] We are indebted to Dr Frank L. Pyman for the α glycerophosphate used in these experiments.

"Acid" phosphatase activity of the serum in diseases other than carcinoma of the prostate gland Included in Table IV are representative

TABLE IV

' Acid and ' alkaline" phosphatase activity of the serum in miscellaneous diseases

Case number	Sex	Age	Serum phosphatase activity		Diagnosis and remarks
			pH 4.9	pH 8.6	
		years	units per 100 cc.	Bodansky units per 100 cc.	
21	♂	55	3 4	112.5	Advanced Paget's disease
22	♀	63	5 0	79.1	Advanced Paget s disease
23	♀	65	1.5	38.0	Advanced Paget s disease
24	♂	49	1.8	10.3	Paget s disease of pelvis, spine
25	♀	50	2.3	30.3	Hyperparathyroidism
26	♂	1	1.9	15.0	Rickets
27	♀	26	1.0	8.3	Osteomalacia
28	♀	15	0.3	3.9	Myositis ossificans
29	♂	65	1.6	33.7	Carcinoma of head of pancreas, obstructive jaundice
30	♂	73	1.2	39 1	? Carcinoma of head of pancreas, obstructive jaundice
31	♂	55	2.3	18.7	Stone common duct, obstructive jaundice
32	♂	60	1 7	11.6	Stone common duct, obstructive jaundice
33	♀	65	1.0	63.6	Biliary cirrhosis, jaundice
34	♂	19	1.6	11.4	Arsphenamine hepatitis, jaundice
35	♂	23	1.1	7.6	Catarrhal jaundice
36	♂	53	1.4	2.3	Hemolytic jaundice
37	♂		1.2	9...	Chronic nephritis, nonprotein nitrogen 150
38	♂	39	2.7	4.4	Uremia, nonprotein nitrogen 123
39	♂	57	1.2	4.4	Multiple myeloma
40	♂	50	0.5	8.8	Multiple myeloma
41	♂	69	1.0	3.4	Lymphatic leukemia
42	♂	56	1.3	12.5	Lymphosarcoma
43	♂	60	2.5	6.4	Osteogenic sarcoma?
44	♂	20	0.6	14.8	Ewing's tumor extensive bone involvement
45	♀	44	1.8	9.8	Carcinoma of breast, extensive osteolytic metastases
46	♀		1...	12 4	Carcinoma (primary?), extensive osteolytic metastases
47	♀	72	1 6	23.3	Carcinoma (primary?) extensive metastases
48	♀	43	1.8	7.7	Hypernephroma, extensive osteolytic metastases
49	♂	70	1.8	33.3	Carcinoma of sigmoid, liver metastases
50	♂	71	0.6	4.9	Carcinoma of stomach, metastases
51	♂	60	1.6	9 7	Seminoma, extensive metastases
52	♂	65	0 6	2.3	Carcinoma of rectum, resected, ? metastases
53	♂	57	1.2	12.3	Carcinoma (primary?), liver metastases
54	♂	74	1.1	9.1	Carcinoma of gallbladder liver metastases
55	○	66	2.6	27 4	Primary

was avoided on the present preparations (1)   Francis (12) showed that both the acetylated and the deacetylated forms of the Type I polysaccharide were capable of stimulating the production of homologous antibody in normal human subjects after 3 intracutaneous injections of 0 01 mgm given one week apart   Felton and Prescott also found the antigenicity of Type I polysaccharides to be independent of the acetyl group (29)

In the various studies mentioned, the materials were obtained from Types I, II, and III pneumococci and were usually given in single or repeated intracutaneous injections of 0 01 mgm to 0 05 mgm amounts   The Types I and II preparations gave the most characteristic reaction, the Type I preparations were most regularly antigenic and the Type III preparations gave the most irregular results   Felton, Sutliff, and Steele (30) gave 2 0 mgm doses subcutaneously using a number of soluble antigens derived by various methods from Types I and II pneumococci   Local reactions consisting of swelling and redness occurred at the site of the subcutaneous injections and usually began to subside in 48 hours   In contrast to the findings of other workers, who elicited only a homologous type specific antibody response, they demonstrated, in human subjects, an increase in protective titer of the serum for both the homologous and the heterologous type, a finding similar to that recorded by Day (31) in rabbits

With a highly purified specific carbohydrate of Pneumococcus Type VIII, Finland and Ruegsegger (32) obtained high titers of the homologous antibody with great regularity by injecting 1 0 mgm subcutaneously   Various preparations of Type III pneumococcus polysaccharide given in the same manner produced antibodies for the homologous type with less regularity and in lower titers   Occasional subjects developed antibodies against the heterologous but related type (33), after receiving either the Type III or the Type VIII carbohydrates   Ruegsegger and Finland (34) also investigated the antibody stimulating efficacy of various doses of Type VIII polysaccharide when given by different routes and found the optimum dose to be 1 0 mgm given subcutaneously   When smaller doses were used, however, they were more effective when given intracutaneously

MATERIALS AND METHODS

The pneumococcus polysaccharides were prepared and furnished in dry form by Dr   Rachel Brown of the Division of Laboratories and Research, New York State Department of Health   The analytical data concerning these preparations were also furnished by her and are given in Table I   Stock solutions of these preparations were made up with sterile 0 85 per cent sodium chloride solution to contain 4 mgm per cubic centimeter

The subjects included normal young adults from the hospital or laboratory staff, medical students, and adult hospital patients without recent febrile illnesses   For the subcutaneous injections, 1 mgm of polysaccharide contained in 1 cc of saline was injected under the skin overlying the deltoid muscle   For the intracutaneous in-

TABLE I

Analytical data concerning the polysaccharide preparations used *

Type	Preparation number	Total nitrogen	Amino nitrogen	P	Acetyl	Ash	Moisture
		per cent	per cent	per cent	per cent	per cent	per cent
I	14	5 34	0 35	0 07	8 17	4 20	6 28
IV	1	4 99	0 18	1 05	12 08	5 84	10 48
V	7	5 37	0 07			3 93	5 94
VII	7	5 59				2 84	6 96
XIV	1	2 83	0 04	0 24	5 26	1 23	10 65

* These materials and the data concerning them were supplied by Dr   Rachel Brown
The percentages are given for the weight of the substances including the moisture

jections, 0 1 cc of solution containing 0 01 mgm of carbohydrate was given into the skin of the flexor surface of the forearm, and this was controlled with a similar injection of the same freshly prepared sterile physiological saline as was used in making the required dilution   No preservative and no heat were employed but the solutions used were cultured and found to be sterile.   Notes were made of the local and general reactions to the subcutaneous injections   The intracutaneous injections were read at one-half hour and again at 18 to 24 hours and were observed at other intervals when indicated   Most of the cutaneous reactions noted with the present materials were similar to those already described (2, 5, 9, 10, 18) except in degree.   They varied in frequency and intensity with the different preparations   Some of the unusual reactions will be considered with each, in turn

Venous blood for serological tests was obtained from each subject before and 10 to 12 days after the injection and occasionally at other intervals   Agglutination tests were carried out with equal volumes of serum dilutions and of formalinized suspensions of actively growing pneumococci containing approximately 1 billion diplococci per cubic centimeter   These were incubated for 2 hours at 37° C. and read after storage in the ice box overnight   The highest dilution of serum showing floccular agglutination was considered the end-point.   Protection tests were carried out with 0.2 cc. of serum, and decimal dilutions of culture injected simultaneously   The virulence of the cultures was maintained by daily mouse passage.

Results with Type I SSS, Preparation Number 14

The local reactions and the results of the serological tests in 4 subjects injected subcutaneously and in 6 injected intracutaneously are listed in Table II   Briefly, the subcutaneous injections gave only slight local reactions in most instances but were regularly followed by a good antibody response as evidenced by the positive immediate

TABLE II

Response to injection of soluble specific substance
Type I, Preparation Number 14

Subject	Sex	Age	Date	I SSS Injected		Local reaction		Type I Antibody titer	
				Amount	Route	Immediate	Delayed	Agglutinins	Protection
		years		*mgm*					
J B T	M	28	June 10	1.0	s.c.	+	±	0	0
			21	0.01	i.c.	++	0	1:4	1,000,000
A. K.	F	69	June 9	1.0	s.c.	++	0	0	0
			21	0.01	i.c.	+	0	1:4	1,000,000
J B	M	28	June 10	1.0	s.c.	+	+	0	0
			21	0.01	i.c.	+	+	1:4	1,000,000
T H H	M	32	June 10	1.0	s.c.	+	±	0	0
			21	0.01	i.c.	++	0	0	1,000
H C*	F	30	May 7	0.01	i.c.	+	+	0	1,000
			17					0	1,000
I M*	M	30	May 7	0.01	i.c.	++	0	0	1,000
			18	—				0	100,000
O L†	M	30	May 10	0.01	i.c.	++	0	0	100
			20	—				0	100
E.G	F	30	May 21	0.01	i.c.	0	0	0	0
			31	0.01	i.c.	0	0	0	100
M W	F	60	May 21	0.01	i.c.	0	0	0	0
			31	0.01	i.c.	0	0	0	0
S S.	F	50	May 21	0.01	i.c.	0	0	0	10
			31	0.01	i.c.	+	0	1:8	1,000,000

* These 2 subjects were healthy carriers of Type I pneumococci 10 days prior to the intracutaneous injection (cf 35)
† Carrier of Type II pneumococci 2 weeks previously

Explanation of Tables II to V inc.

Route s.c. = subcutaneous; i.c. = intracutaneous.
Local reactions
Following subcutaneous injections (delayed)
± = tenderness only
+ = tenderness and swelling less than 2 cm.
++ = redness, tenderness, swelling more than 3 cm., or with constitutional symptoms.

Immediate intracutaneous reactions
± = wheal larger than control without pseudopods, but with surrounding erythema.
+ = wheal well defined with pseudopods and bright erythema 2.0 cm. or more.
++ = edematous wheal more than 1.5 cm definite pseudopods and bright erythema more than 3.0 cm.

Delayed intracutaneous reactions (8 to 24 hours)
± = 0.5 to 1.0 cm redness with slight tenderness.
+ = 1.0 cm. or greater redness and tenderness.
++ = local edema 3 cm. or more.
Agglutinins 1 2 4 etc. = highest serum dilution showing floccular agglutination.
Protection largest number of fatal doses which mice survived with simultaneous injections of 0.2 cc of serum.
— = Not done.
I V etc. = Type I Type V etc.
SSS = soluble specific substance.

'Tests for antibodies against heterologous types of pneumococci were done on the sera of 5 subjects, using Types II, V, and VIII pneumococci No antibodies developed against these types

Results with Type V SSS, Preparation Number 7

This material was given to 5 subjects subcutaneously and to 6 intracutaneously The data for these subjects are given in Table III The

TABLE III †

Effect of injection of soluble specific substance
Type V, Preparation Number 7

Subject	Sex	Age	Date	V SSS Injected		Local reaction		Type V Antibody titer	
				Amount	Route	Immediate	Delayed	Agglutinins	Protection
		years		*mgm*					
T J F	M	65	June 9	1.0	s.c.	+	0	0	0
			19	0.01	i.c.	0	0	0	0
			July 30					0	1,000
W R.	M	43	June 9	1.0	s.c.	0	0	0	0
			19	0.01	i.c.	0	0	1:2	100,000
			July 30					1:2	10,000
F J P	M	31	June 9	1.0	s.c.	++	++	0	0
			21	0.01	i.c.	+	+	0	1,000
			July 27					0	1,000
J W B	M	26	June 9	1.0	s.c.	+	++	0	0
			21	0.01	i.c.	+	++	1:8	1,000,000
			July 27					1:8	1,000,000
L. O'N	F	33	June 9	1.0	s.c.	0	0	0	10
			19	0.01	i.c.	0	0	0	10,000
			July 30					0	100,000
L. J*	M	28	May 11	0.01	i.c.	±	0	0	0
			20					0	100,000
H C.	F	29	May 7	0.01	i.c.	+	+	0	1,000
			17					0	1,000
I J M	M	30	May 7	0.01	i.c.	++	0	0	100
			18					0	1,000,000
M D	F	48	May 22	0.01	i.c.	0	0	0	0
			June 1	0.01	i.c.	0	0	0	100,000
J A	M	60	May 19	0.01	i.c.	0	0	0	0
			May 28	0.01	i.c.	0	+	0	0
W D	M	28	May 19	0.01	i.c.	0	0	0	1,000
			29	0.01	i.c.	0	0	0	10,000

† See Table II for explanation of symbols.
* Carrier of Type VIII pneumococci on April 27, serum protected against 1 000 000 fatal doses Type VIII pneumococci on that day (cf 35)

cutaneous reaction and the high titer of mouse protective antibodies A comparable response to the single intracutaneous injection was obtained in only one subject. The others developed antibodies of low titer or increased previously existing levels only slightly if at all

type-specific antibody response to this preparation was not as good as in the case of the Type I material, and the local reactions were irregular Positive cutaneous reactions did not correlate with the antibody findings Some of the subjects who showed no protective antibody 10 and 12 days respectively, after the subcutaneous injection later developed such antibodies presumably aided by the additional stimulus of the intracutaneous injection used for the tests Agglutinins appeared in 2 subjects only, and they had a rela-

tively high titer of protective antibody in the control serum

Tests for protective antibody against the related Type II pneumococci (36) were done on all the sera of the subcutaneously injected subjects and none developed antibodies for this type

Results with Type VII SSS, Preparation Number 7

This preparation was given to 5 subjects subcutaneously and to a similar number intracutaneously The former all showed some local reaction to the injection They all later gave strongly positive intradermal tests and, with one exception, developed agglutinins in their sera The latter all failed to show an increase in the homologous antibody titer The results of the various tests are listed in Table IV

TABLE IV †

Effect of injection of soluble specific substance Type VII, Preparation Number 7

Subject	Sex	Age	Date	VII SSS Injected		Local reaction		Type VII Antibody titer	
				Amount	Route	Immediate	Delayed	Agglutinins	Protection
		years		*mgm*					
W B	M	28	June 10	1 0	s.c	++	+	1 4*	
			21	0 01	l c		+	1 32	
M L	M	24	June 10	1 0	s c	++	+	1 8*	
			21	0 01	l c		0	1 8*	
M T	M	24	June 10	1 0	s c	++	+	0	
			21	0 01	l c		0	1 4	
J M H	M	30	June 9	1 0	s.c	++	±	0	
			21	0 01	l c		0	1 16	
			July 28	—				1 16	
R W H	M	28	June 10	1 0	s c	++	++	0	
			21	0 01	l.c		±	1 8	
			July 28	—				1 4	
C G	M	64	April 15	0 01	l c	±	0	1 2	100 000
			24	—				1 2	10 000
C V	M	54	May 18	0 01	l c	++	0	1 2*	1 000 000
			28	0 01	l c			1 2*	1 000 000
E C	M	21	May 18	0 01	l c	0	0	0	100 000
			28	0 01	l c			0	1 000
W D	M	48	May 18	0 01	l c	+	0	1 2*	100 000
			28	0 01	l c	0	0	0	100 000
A F	M	57	May 18	0 01	l c	+	0	1 4	1 000 000
			28	0 01	l c	+	+	1 4	10 000 000

* Fine floccular agglutination
† See Table II for explanation of symbols

Mouse protection tests with the Type VII pneumococcus and human sera have not been entirely satisfactory because of the irregular virulence of this organism and the high titers of protection found in the sera of most normal individuals, and even during the acute stage of pneumonia due to this type (37) *Previous studies*

indicated that the protection test is a more delicate index of the development of small amounts of specific antibody than either the agglutination test or the cutaneous reaction to type-specific polysaccharides (4) In the present study, therefore, Type VII protection tests were carried out only with the sera of the intracutaneously injected subjects All showed protection in the control sera but no significant increases in titer appeared in the later ones

The sera of each of the subjects were tested for the development of antibodies against one or more heterologous types of pneumococci Types I, II, IV, V, and VIII pneumococci were used in agglutination and mouse protection tests and all yielded negative results

Results with Type IV SSS, Preparation Number 1 and Type XIV SSS, Preparation Number 1

Two series of observations were made with these preparations In one group, each subject was given a subcutaneous injection of one of the preparations This was followed in about 2 weeks by intracutaneous tests with both preparations, and the blood was studied just before and about 3 weeks after these intradermal tests to determine the antigenic effects of both the subcutaneous injection and of the skin test injections The data for this group are shown in Table VA In the second group, one of the preparations was given intracutaneously and skin tests were done later with both The results in this group are given in Table VB

Briefly, the Type IV preparation gave local reactions regularly when injected subcutaneously and frequently to an initial intracutaneous injection, whereas the Type XIV preparation was free of such reactions Both preparations gave nonspecific reactions to later injections Both gave good antibody responses for the homologous pneumococcus type The subcutaneous and intracutaneous injections were about equally effective *Protection tests were not done with the Type XIV pneumococcus because none of the strains available could be raised in virulence sufficiently to be satisfactory for this purpose

Agglutinins and mouse protection tests were also carried out with Types II, V, VIII, and XI pneumococci on these sera Only 3 subjects developed protective antibody in their sera against

...eterologous pneumococcus types Subjects T D ind W C developed protection against 10,000 ind 100 lethal doses, respectively, of Type II pneumococci and Subject M McR. developed protection against 100,000 fatal doses of Type V pneumococci

An unusually severe reaction to the intracutaneous injection of Type IV SSS was observed in Subject J V (Table VA) A yellow edematous wheal appeared almost immediately and increased for 40 minutes to a maximum diameter of 2.5 cm. It had numerous stubby pseudopods and was surrounded by an area of intense erythema 8.5 cm. in diameter The wheal gradually blended with the surrounding erythema to form a soft puffed up area elevated about 2.5 cm. in the center which involved half the flexor surface of the forearm and was exquisitely tender There was no redness or lymphangitis visible, but one of the axillary nodes became enlarged and tender The entire reaction subsided in about 24 hours and it was not accompanied by any febrile reaction The initial subcutaneous injection of the same material had given rise to a red tender swelling about 6 cm. in diameter In a second subject, G P (Table VB) the second intracutaneous injection of the same material gave a very similar reaction except that an epitrochlear node was enlarged in this instance.

TABLE V †

Effect of injection of Type IV SSS Preparation 1 and Type XIV SSS Preparation 1

A Subcutaneous injection of one followed by intracutaneous injection of both

Subject	Sex	Age	Date	Type IV SSS injected — Amount	Route	Immediate	Delayed	Type XIV SSS injected — Amount	Route	Immediate	Delayed	Agglutinins Type IV	Agglutinins Type XIV	Protection Type IV
		years		*mgm.*				*mgm.*						
A B	F	63	June 26 July 6 Aug 13	1 0 0 01	s.c. i.c.	0	++ 0	— 0 01	i c	0	0	0 0 0	0 0 1 2	0 0 0
J C.*	M	44	June 26 July 6	1 0	s c.		+	—				0 1 64	0 0	0 100 000
H L.*	M	51	June 24 July 6 Aug 3	1 0	s.c.		+	—				0 1 4 1 4	0 1 : 4 1 4	0 10 000 1 000
J V	F	48	June 24 July 6 July 31 Nov 9	1 0 0 01	s c. i c.	++	++ ++	— 0 01	i c.	±	0	0 1 64 1 32 1 32	0 0 1 16 1 8	0 100 000 1 000 000 1 000 000
W C.	M	51	June 24 July 6 July 29	1 0 0 01	s c i.c.	++	++ ++	0 01	i c.	+	0	0 1 8 1 32	0 0 1 2	0 1 000 000 10 000 000
R. E. M	M	28	June 24 July 7 July 27	— 0 01	i c	+	0	1 0 0 01	s c. i.c.	++	0 ++	0 0 1 8	0 1 32 1 32	100 100 1 000 000
T D	M	27	June 28 July 10 July 27	0 01 0.01	i c	++	++	1 0 0 01	s.c. i c	++	0 ++	0 0 1 2	0 1 2 1 2	0 10 100 000
M McR.	M	27	June 24 June 7 July 27	0 01	i.c	±	0	1 0 0 01	s.c. i.c.	+	0 0	0 0 1 16	0 1 16 1 8	1 000 1 000 1 000 000
F E.	M	28	June 24 July 7 July 27	0 01	i.c.	0	0	1 0 0 01	s.c. i c	0	0 0	0 0 1 4	0 1 4 1 4	0 0 100 000
H S	M	26	June 24 July 9 Aug 4 Oct. 26	0 01	i c.	+	0	1 0 0.01	s c. i.c.	+	0 0	0 0 1 8 1 16	0 1 16 1 16 1 32	0 0 10 000 100 000

TABLE V—*Continued*

B Intracutaneous injection

Subject	Sex	Age	Date	Type IV Immediate	Type IV Delayed	Type XIV Immediate	Type XIV Delayed	Agglutinins Type IV	Agglutinins Type XIV	Protection Type IV
				Reaction to 0 01 mgm SSS injected intracutaneously				Antibodies		
Г R R	M	41 *scars*	July 28	0	0	—	—	0	0	0
			Aug 9	+	++	±	0	1 16	0	10,000,000
G P	M	38	July 28	0	0	—	—	0	0	0
			Aug 9	+	++	±	+	1 16	1 2	1,000,000
F W	M	68	July 28	0	0	—	—	1 2	1 2	10,000
			Aug 9	0	+	0	+	1 4	1 2	10,000
J J	M	70	July 29	++	0	—	—	0	0	100,000
			Aug 9	++	+	±	0	1 32	0	10,000,000
A D	M	46	July 29	±	+	—	—	0	0	0
			Aug 9	0	+	0	0	0	0	1,000
J C	M	46	July 28	0	0	—	—	0	1 4	0
			Aug 9	0	+	0	0	1 2	1 2	100,000
M McN	Г	58	July 30	—	—	0	0	0	0	0
			Aug 9	0	0	+	++	0	1 16	0
V L	F	60	July 30	—	—	0	0	0	0	0
			Aug 9	±	0	++	+	0	1 4	0
M D	M	36	July 29	—	—	0	0	0	0	10
			Aug 9	0	0	0	0	0	1 4	10
H S	M	68	July 30	—	—	0	0	0	0	0
			Aug 10	+	0	+	0	0	0	0
E S	M	24	July 29	—	—	0	0	1 2	0	10,000
			Aug 10	+	0	0	0	1 4	1 2	10,000
F F	M	78	July 29	—	—	0	0	0	0	0
			Aug 10	0	+	0	0	0	1 4	10
W J	M	37	July 30	—	—	0	0	0	0	0
			Aug 9	+	±	0	0	0	0	0

* No intracutaneous tests
† See Table II for explanation of symbols

The results of all the tests are summarized in Table VI

Single skin tests in normal subjects

Tests with 0 01 mgm of each of the preparations were made in 25 other subjects not listed in the previous tables   Each subject was tested with one of the preparations and the serum tested for the homologous antibody   The results are summarized in Table VII   There was no correlation between the positive tests and the corresponding antibody

DISCUSSION

The various studies previously cited indicate that in human subjects, just as in animal experiments, significant differences may be observed in the response to injections of polysaccharides derived in different ways from the same type of pneumococcus, and, conversely, preparations which differ chemically and immunologically may elicit similar responses in human subjects (*cf* 9, 12)

The materials used in the present study were obtained by methods designed to avoid the use of

TABLE VI

Resumé of local reactions and circulating antibodies resulting from injection of 5 polysaccharides

Material					Local reactions *				Homologous antibody response †							Heterologous antibody		
					Initial		Subsequent		Agglutinins			Protection						
Type	Preparation	Amount	Route	Number of subjects	Immediate	Delayed	Immediate	Delayed	0	2 or 1:4	+8 or 1	0	10 or 100	1,000 or 10,000	100,000 or more	Number of tests	Number positive	Type
I	14	1 0	s.c	4		3	4	2	1	3	0	0	0	1	3	6	0	
		0 01	i.c.	6	3	1	1‡	0	5	0	1	2	3	0	1	4	0	
V	7	1 0	s.c.	5		3	3	2	3	1	1	2	2	1	0	8	0	
		0 01	i.c	6	3	2	0	0	6	0	0	1	2	1	2	5	0	
		0 01	i.c.	5§	3	2			5	0	0	2	1	2	0			
VII	7	1 0	s.c.	5		5	5	2	1	1	3					10	0	
		0 1	i.c.	5	4	1	2	1	5	0	0	4	1	0	0	5	0	
IV	1	1 0	s.c.	5		5	2	2	1	1	3	1	0	1	3	19**	1	II
		0 01	i c	6	2	1	3	6	1	2	3	1	1	1	3	6**	0	
		0 01	i.c.	5‖	4	1			0	2	3	0	0	4	1			
XIV	1	1 0	s c.	5		0	4	2	0	2	3					12††	2	II V
		0 01	i c.	7	0	0	3	2	2	4	1					7††	0	
		0 01	i.c.	3¶	2	0			0	2	1							

* To the initial subcutaneous or intracutaneous injection and the subsequent intracutaneous test with the same preparation

† Titer acquired or increased Antibodies listed with the subcutaneously injected subjects represent those demonstrated before any later intracutaneous injections of the same materials

‡ Only 3 whose initial reactions were negative were retested

§ Each of these had previously received 1 0 mgm. of the same material s.c.

‖ After 1 0 mgm. Type XIV SSS given s.c.

¶ After 1 0 mgm. Type IV SSS given s c.

** Skin tests done in 9 subjects with Type XIV SSS 5 immediate positive and 2 delayed positive reactions.

†† Skin tests with Type IV SSS done in 12 subjects 8 gave immediate and 3 gave delayed reactions.

heat and of chemical reagents that alter the character of the products (1) Of the 5 preparations used, 4 were specific polysaccharides of types not previously tested in human subjects, namely Types IV, V, VII, and XIV (38), and the fifth was a Type I preparation The local reactions to initial intracutaneous injections observed with all of these preparations were similar to those observed by Finland and Dowling (9) with the so-called cellular carbohydrates Immediate and delayed reactions occurred together or independently of one another or of the presence of homologous type-specific antibody in the circulating blood. In the small numbers of subjects tested the intracutaneous reactions, immediate and delayed but particularly the latter appeared more frequently after previous injections of the same or of some other polysaccharide had been given subcutaneously than with the initial intracutaneous injection. These reactions were not associated with homologous type specific antibodies The findings suggest that these materials contain a non-type specific antigen in addition to the type specific component

The present observations with different doses and routes of injection and similar studies of other investigators (19, 23, 30, 34) indicate that the optimum dose may vary widely with different preparations of each polysaccharide—that toxic effects or immunity may arise only within a certain range of dosage This optimum must, therefore, be worked out with each material and for each animal species Improper dosage or route or intervals may account for the irregular response obtained with some of the materials

The antibodies demonstrated following the injection of the various specific carbohydrates used in this and in most of the other studies mentioned were almost wholly specific for the homologous type of pneumococcus Only rare subjects de

tity in the liver and spleen, and to a lesser extent in the kidney and skin after the administration of large amounts of iron

The absence of demonstrable increase of iron in the gastro intestinal tract under the conditions of this experiment fails to show that the large intestine is the site of iron excretion, and corroborates the recent findings of other workers in the field of iron metabolism, namely, that iron is not excreted to any appreciable degree by the intestinal tract

The Relationship between the Fractions of the Serum Protein Complex By HENRY TIFID, JR, and (by invitation) DANIEL MELNICK and CHRISTOPHER PARNALL, JR, Ann Arbor, Mich

There has been produced much evidence to indicate that the serum protein complex is composed of two or more unstable coprecipitation systems in mutual equilibrium and that the protein fractions isolated by physicochemical methods are not preexistent Variations in the concentrations of the isolated fractions are considered to result from disturbances in the balance of the component systems

We have dialyzed normal sera against nephrotic sera, characterized by hypoproteinemia and markedly low albumin to globulin ratios, for variable periods of time and at selected temperatures In this manner the normal and nephrotic serum proteins were subjected to practically the same environmental influence with respect to concentration of protein and of the crystalloids, pH, and temperature Nevertheless, no change in the albumin to globulin ratios of either the normal or nephrotic sera resulted Serum lipids were observed to exert no appreciable influence upon the salting out of the protein fractions

These studies are not interpreted as indicating that such protein fractions are preexistent in serum They seem to indicate that any association or dissociation of protein molecules must occur within independent systems and that fractionation obtained by precipitation methods is different from that obtained by ultracentrifugation The reason for the change in ratio of precipitation fractions in pathological states remains to be explained

A Long Term Study of the Variation of Serum Cholesterol in a Group of Relatively Normal Individuals By KENNETH B TURNER and (by invitation) ALFRED STEINER, New York, N Y

Although there are innumerable reports on the blood cholesterol of man under normal or abnormal conditions, the vast majority of these are based on single or, at best, a few determinations in a given individual Little is known of the normal variation from week to week over long periods under controlled conditions

An unusual opportunity was afforded to study the serum cholesterol of 11 relatively normal but hospitalized patients at approximately weekly intervals for a year or more. Frequent determinations of the basal metabolic

rate and other factors that might influence the serum cholesterol were made.

The constancy for the individual of the cholesterol level in his serum was established Thyroid extract produced a marked fall in the serum cholesterol High or low fat diets, the administration of cholesterol or of potassium iodide were without effect

Furthermore, no significant variations in serum cholesterol occurred during the course of a day The futility of the so-called "cholesterol tolerance tests" was demonstrated

Allergic Bronchial Obstruction and Bronchiectasis By HERMAN H RIFCKER, Ann Arbor, Mich

Bronchiectasis has been due, in occasional cases, to extrinsic pressure on a small bronchus collapsing its lumen, to cancer, or to fibrosing and inflammatory lesions which obstruct the bronchus The common factor in these cases is an obstruction with trapping of the *secretion and consequent infection*

In searching for a cause more applicable to the large majority of cases, it seemed advisable to investigate the possible relationship of bronchiectasis to allergic disease of the respiratory tract

Pathological studies, particularly by Alexander, have shown that in asthma, bronchial obstruction does occur at about the 5 mm level, and recent lipiodal studies here confirm this finding for the active case It is not a diffuse process and is produced mostly by edema and mucus Below this level there often is dilatation in the walls of *the bronchioles with the occasional formation of bullae and emphysema* Eosinophilic infiltration of muscle and degeneration of cartilage are observed In autopsy material of bronchiectasis, eosinophiles in the wall of the bronchus above the infected area have been seen, and it has not been uncommon to find many eosinophiles in the bronchiectatic sputum when proper search is made

The hospital records of 122 consecutive cases, diagnosed as bronchiectasis by bronchograms, and containing the results of an examination of the nasal membrane by competent otologists, were studied with respect to the presence of allergy, with the following results

Cases of bronchiectasis with

Asthma only	5
Allergic rhinitis only	68
Asthma and allergic rhinitis	22
Neither	27
	122

Thus 77 8 per cent of the cases of bronchiectasis appear to have occurred in individuals with allergic respiratory tract disease which long antedated the symptoms of bronchiectasis In the remaining cases there was no proof of allergy, possibly because in some cases the allergic changes were reversible and not constantly present in the nasal membrane

Bronchoscopic observations in our clinic have shown that in allergic rhinitis, and in many cases of bronchiectasis, the allergic edema is by no means limited to the

nasal membrane but may extend quite far down the individual bronchi without producing the symptom of asthma. In bronchiectasis complete obstruction of individual bronchi of the third order has been seen to disappear after the application of adrenalin and cocaine. Similarly, it has been observed that in asthma the nasal membrane frequently is involved in the allergic process.

In view of these observations it is not unreasonable to suppose that the allergic bronchial reaction associated with allergic rhinitis produces partial or complete obstruction with distal dilatation and weakening of the bronchial wall to form the basis for many cases of bronchiectasis The purulent upper respiratory infection (present in 72 per cent of the cases) may be a contributing or activating factor but not a primary one. This conception of the basic etiology of the disease offers some hope for its anticipation and prevention in the allergic child. In the treatment of the disease by drainage, the factor of edematous obstruction may well be taken into consideration.

Cytological studies for eosinophiles in material obtained by bronchoscope from the bronchiectatic cases are being continued.

Studies in Tuberculous Calcification By ROBERT G BLOCH Chicago Ill.

Calcification, besides resorption, is the only healing process of the tuberculous lesion itself. The deposition of calcium salts sufficient to be clinically recognized as calcification is a very slow process. The clinical conception of calcification is based exclusively on its roentgenological appearance, the degree of density being the chief criterion. The grossly calcareous lesion represents the desired final stage of healing and is erroneously confused with the degree of calcification, i.e with the amount of calcium deposited. While the amount of calcium expresses itself roentgenologically the calcareousness does not. Involvements with a very high calcium content may still be softly caseous and are potential excavations of clinical importance. The size of a lesion is a better diagnostic guide than the roentgenologic degree of density

Small tuberculous lesions in the lung with certain characteristics as to size, location, consistency chemical content, and roentgenological appearance, are universally accepted as evidence of primary "childhood" infection. From the studies of Ghon in children the conclusion was reached, without sufficient evidence, that such lesions, when found in adults, signify the remains of an infection contracted in early life.

Without making a definite contention that the existing conception is erroneous, the question is opened in this paper if many of the so-called primary tuberculous foci do not develop during adult life. Some clinical evidence is presented tending to show that they may be another form of tuberculosis developing in adults as the result of exogenous superinfection. The initial results of an experimental study comparing roentgenological, histological and chemical factors are discussed.

Induction of Lymphoma by Carcinogenic Agents By AUSTIN M. BRUES and (by invitation) BEULA B MARBLE, Boston Mass.

A study has been made of the effect of cancer producing agents on animals with a constant low susceptibility to lymphoma. One hundred and eighty mice were used of a special strain in which the normal incidence of lymphoma is approximately 2 per cent, and the incidence was markedly increased by the application of carcinogenic tar to the skin. Three batches of tar were used, of widely varying degrees of carcinogenic potency, and the incidence of lymphoma followed, pari passu the potency of the tar, reaching 50 per cent in the group receiving the most actively carcinogenic agent. The lesions produced were characteristic, involving spleen, lymph nodes and usually liver with an associated subleukemic blood picture. The disease in control animals runs a relatively benign course of several months while in tarred animals it progresses rapidly and they usually die within a few weeks of the clinical onset. Although cancer producing agents do not readily produce lymphoma in other strains of animals these results suggest that in the presence of a latent predisposition they may influence its development and course.

The Entrance of Proteins into Joints and Certain Other Body Cavities By GRANVILLE A. BENNETT and (by invitation) MORRIS F SHAFFER, Boston, Mass.

Previous studies have demonstrated that foreign proteins injected intra-articularly are removed from joints solely by way of the lymphatics, whereas drugs in aqueous solution are removed from joints chiefly by way of the blood capillaries. The purpose of the present experiments was to study the transference of proteins from the blood stream into joints Information so obtained should lead to a better understanding of normal joint physiology and the mechanism involved in the production of joint effusions. In addition, the passage of such substances into the aqueous chambers and the sub arachnoid space was investigated.

Crystalline egg albumen or horse serum protein fractions were injected intravenously into rabbits. By employing the precipitin test with specific antisera as means for their detection, these proteins could be demonstrated to have passed regularly within a short of time, from the blood stream into the knee joint They also appeared in the aqueous chambers of eyes, but in lower concentration. In most no foreign protein was detected in the spinal fluid present its concentration was considerably lower in the joint washings or aqueous fluids.

Such data concerning the entrance into and resid in various body cavities of foreign proteins should as a basis for experimental studies on tissue sitivity and the treatment of infections of joints specific antisera.

Unequal Distribution of Respiratory Gases in Emphysematous Subjects, its Measurement and Significance By A Cournand and J S Mansfield (by invitation) and D W Richards, Jr, New York, N Y

Several different techniques were employed, with normal and emphysematous subjects, to test the state of mixture of intrapulmonary gases, both before and during the course of quiet breathing in a small closed circuit consisting of lungs, spirometer, soda lime container, and connecting tubing Varying factors in the technique were 1, (a) preliminary breathing of pure oxygen for ten minutes before onset of rebreathing, or (b) preliminary air breathing, 2, variation in composition of gases in spirometer before rebreathing, 3, (a) steadily decreasing volume of closed breathing circuit, or (b) maintenance of constant volume of lung-spirometer circuit. With each of these methods it is possible, after certain corrections are made, to calculate pulmonary residual air values, according to the principle of nitrogen dilution

In normal subjects all techniques gave essentially the same values for functional residual air

In certain emphysematous subjects, washing out of the lungs by pure oxygen breathing, preliminary to the closed circuit breathing, gave residual air values much lower than the (usually large) values found after preliminary air breathing This suggests that the resting emphysematous lung contains excess nitrogen, presumably in the large hypoventilated but still perfused pulmonary spaces

The discrepancy between the residual air values obtained by these different preliminary breathing procedures can be used as a quantitative measure of unequal distribution of respiratory gases in pulmonary spaces The large values found for residual air in emphysematous subjects are in some cases due in part to the same phenomenon Such residual air figures in these cases may be considered as a combined index of physiological dysfunction, rather than as a measure of strictly anatomical volume

The Inhibition of Choline Esterase of Muscle by Prostigmine with Reference to the Action of the Drug in Myasthenia Gravis By William C Stadie and (by invitation) Maxwell Jones, Philadelphia, Pa

Recently prostigmine has been shown to ameliorate dramatically but briefly the symptoms of myasthenia gravis In explanation, the hypothesis has been advanced in the literature that the disease is associated with a high content of muscle choline esterase. In consequence, acetylcholine, when formed, is destroyed so rapidly that the ordinary neuro-humoral mechanism of nerve-muscle transmission is impaired and symptoms of the disease result. According to this view, prostigmine produces its effect by partial inhibition of the esterase of the muscle thus restoring to normal the neuro-humoral mechanism.

No muscle esterase values in myasthenia cases have

been reported and the serum esterase values are f be within normal limits However, the association marked inhibition of serum esterase and the 1 of the symptoms by prostigmine still lends color to hypothesis

We have studied the effect of prostigmine upon esterase of serum and muscle of guinea pigs, n humans, and in one case of myasthenia gravis T cases the results are essentially the same and summarized as follows

In guinea pigs, the intravenous injection of or prostigmine in doses which are comparable to peutic doses in humans resulted in marked inhib the choline esterase activity of the serum In however, we were unable to show any inhibition ever Even with toxic doses no inhibition shown The following is a typical protocol

The choline esterase activity of muscle and serum guinea pig before and after the intravenous inje of 10 mgm of eserine (activity in μM per gra per minute of acetylcholine hydrolyzed)

	Muscle	Serum
Before	0 68	0 66
After	0 69	0 20

When muscle or serum is equilibrated in vit known concentrations of prostigmine it was f whereas serum esterase is markedly inhibited, t little or no inhibition of muscle esterase b at concentrations within the therapeutic zone On the concentration of the prostigmine greatly the therapeutic concentration could inhibition be strated, and in all cases this was much less case of the serum The following is a typical in the guinea pig

Choline esterase activity of guinea pig muscle when exposed in vitro to known concentrations prostigmine Results are expressed as a the value in the absence of prostigmine

Concentration of prostigmine		Relative
mgm per liter		Muscle
0 0	Therapeutic zone	1 00
0 05	Therapeutic zone	1 00
0 1		0 90
0 5		0 59
1 0		0 32
2 0		0 23

In one case of myasthenia gravis the cho of the muscle (1 63 μM per gram per minute) to be quite comparable in value to that of human subjects (1 60 and 1 95 μM per gram

The behavior of the muscle esterase in this the inhibiting action of prostigmine in vitro to that found in the case of the guinea pig

Relative activity of muscle and serum choline esterase of a case of myasthenia gravis when equilibrated with known concentrations of prostigmine

Prostigmine mgm per liter		Esterase activity Muscle	Serum
0 0	Therapeutic zone	1 00	1 00
0 005	Therapeutic zone		0 90
0 01	Therapeutic zone		0 86
0 05	Therapeutic zone	1 19	0 57
0 1		1 11	0.54
0.5		0 73	0.32
1 0		0 79	0 23
2 0		0 41	

Conclusions 1 The inhibiting effect of prostigmine in the choline esterase of muscle and serum of guinea pigs and humans is quantitatively quite different. Only concentrations quite outside the therapeutic range depress the activity in the case of the muscle and then the effect is much less than in the case of the serum.

2. The muscle esterase content of a case of myasthenia gravis was about the same as that found in two normal human sub ects

3. Our data lend no support to the hypothesis that myasthenia gravis is associated with an unduly high choline esterase of the muscle furthermore, the evidence is against the hypothesis that the beneficial effect of prostigmine is due to its inhibiting action upon the muscle esterase.

Clinical Study of Persons with Subnormal Temperatures By HOBART A REIMANN, Philadelphia, Pa.

Several years ago I collected and studied 16 patients whose temperatures were found to be slightly higher than the accepted normal It was shown that the temperature was not true fever but was normal for these patients Hyperthermia is rather frequently encountered in per f s of a certain type, especially in women in the latter half of the menstrual cycle.

Subsequently I studied several persons all happened to be men, whose temperature averaged below the normal level and varied between 96° F and 98° F Each of these patients came to the hospital with numerous bizarre complaints which are often associated with neurasthenia. The outstanding complaints were weakness, abdominal distress cold and sweaty extremities, and palpitation of the heart. After complete studies were made no impor tant deviations from the normal were found except a tendency to hypothermia, hypotension, and bradycardia. Both the blood pressure and heart rate were quickly raised by physical or emotional stress The basal meta bolic tests were usually within normal limits Some pa tients reacted markedly to small doses (1 mgm.) of pilo carpine or to 0.5 cc. doses of epinephrine solutions. Atro pine, thyroid substance opium benzedrine, pilocarpine

and epinephrine had little or no effect on the temperature. Exercise and emotional excitement sometimes caused a temporary increase in blood pressure and pulse rate.

Each of the patients can apparently be placed either in the group classed tentatively as vagotonia or sympathi cotonia, but the symptoms rarely fit completely into one group or the other

The Use of Trypsin in Producing Experimental Nephri tis By LOUIS N KATZ and (by invitation) MEYER FRIEDMAN Chicago, Ill.

An acute nephritis was produced in dogs by a single injection of a 1 per cent solution of commercial trypsin directly into both renal arteries. The nephritis was characterized by glomerular hemorrhage and inflamma tion, and could be made severe enough depending upon the amount of trypsin injected, to lead to a lasting nephritis terminating in uremia Control injections of casein and of lipase into the renal arteries led to no recognizable lesions in the kidneys or changes in their function. We are studying the effects of this trypsin nephritis upon the dog's blood pressure.

The Effect of Sulfanilamide on Electrolyte Metabolism. By WILLIAM W BECKMAN (introduced by James H Means), Boston, Mass.

A reduction of the carbon dioxide combining power of the serum during the administration of sulfanilamide in dicates that a derangement of the electrolyte metabolism takes place. In order to define better this disturbance the acid base excretion and serum concentration of patients receiving sulfanilamide were studied. They were kept on a constant diet and fluid intake.

The first effects noted are a striking increase in the sodium excretion a marked reduction in ammonia out put and a strongly alkaline urine (pH 7 4 to 7 8) The serum sodium concentration falls 5 or 6 meq There oc curs a corresponding diminution in the serum carbon dioxide content. Within a few days the sodium and ammonia excretion and the urine pH return to pretreat ment values despite continuances of the drug The low ered serum sodium concentration, however persists as long as sulfanilamide is given (28 days in one ment) When the drug is discontinued the reverse nomena are observed. Sodium is retained, ammonia ex cretion is increased and the urine becomes acid. T serum sodium concentration returns to its normal level The potassium excretion follows a course similar to sodium but of considerably less magnitude.

In order to define more completely the changes in internal environment of the organism caused by sulf amide, we are making similar observations on electrolytes

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