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# PROCEEDINGS

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# SCIENTIFIC PROCEEDINGS.

## Abstracts of Communications.

### Sixty-ninth meeting.

*Cornell University Medical College, October 20, 1915. President Lusk in the chair.*

1 (1065)

### As to the cause of the dilatation of the subclavian artery in certain cases of cervical rib.—Experimental Study.

By **W. S. Halsted, M.D.** and **Mont Reid, M.D.**

[*From the Department of Surgery, Johns Hopkins University.*]

In twenty-four or more instances a circumscribed dilatation of the subclavian artery has been observed in cases of cervical rib. The dilatation in these cases is distal to the site of pressure made by the rib.

As to the cause of these aneurisms there has been considerable conjecture, usually prefaced by the comment that their occurrence would be comprehensible if they presented on the proximal instead of on the distal side of the compression.

Weakening of the wall of the artery from erosion or trauma, variable or intermittent pulse pressure, and vasomotor disturbances in nutrition are the suggestions which have been offered to explain the phenomenon.

For several years my experiments in arterial compression have had more or less in view the determination of the cause of this dilatation. For the past year they have been continued by Dr. Mont Reid and myself almost exclusively with the object of shedding light on this problem. In 1906 we (Dr. Richardson,

Dr. Dawson and myself) made the observation[1] that after partial occlusion of the thoracic aorta the maximum pressure may be permanently lowered as much as 46 mm. Hg, and the minimum pressure actually increased distal to the constricting band of metal.

The dilatation of the artery observed in arterio-venous fistula, might, it seemed to me, have a bearing on the interpretation of the aneurisms in cases of cervical rib. Might not both phenomena, I asked myself, be due to degenerative changes in the arterial wall consequent upon lowered pressure—in the case of the cervical-rib-aneurisms, upon lowering of the pulse pressure.

Now, inasmuch as dilatation of the subclavian artery has relatively so seldom been observed with cervical rib (perhaps 24 times in about 400 cases) it seemed to me that if it were due merely to the lowered pulse pressure then only a very definite absolute or relative amount of reduction of the systolic pressure would suffice to produce it.

In June, 1914, I observed, in a dog, for the first time an unquestionable dilatation of the three arteries below the constricting band which had been placed just above the aortic trifurcation. The constriction exercised by the band was sufficient to greatly lessen, if not, indeed, to obliterate the palpable thrill produced by the constriction, but not enough to shut off the palpable pulse. With this observation as fresh incentive, Dr. Reid and I have continued the experiments for the past year and a half with encouraging results: in only one additional instance, however, was there a very striking dilatation. In this, as in the one of the preceding year, the occlusion of the aorta by the band was almost total.

If the occlusion must be so nearly complete in order to effect a pronounced dilatation it will assist to explain not only the difficulty we have had in producing it in dogs, but also the fact that it has been observed relatively so seldom in the human subject from compression of the subclavian artery by a cervical rib. For when in dogs the aortic pulse is occluded beyond the stage of

[1] DOG 96. PARTIAL OCCLUSION OF THORACIC AORTA.
Operation, 22/5/1906. Sacrificed 7 months later.

| | Maximum Pressure. | Mean Pressure. | Minimum Pressure. | Pulse Pressure. |
|---|---|---|---|---|
| Femoral | 116 | 93 | 88 | 28 |
| Carotid | 160 | 113 | 83 | 77 |

palpable thrill the lumen is in danger of becoming obliterated—as by the formation of a cylindrical fibrous cord beneath the band—and thus cancel the experiment; and in the cervical rib cases we may assume, argumentatively, that the subclavian artery, compressed to the stage sufficient to produce an aneurism, is likely to become totally occluded in the presumably considerable time required for the manifestation of the dilatation. Thus, in dogs a number of months must apparently elapse after the application of the band before a dilatation in striking degree can occur. In the two cases, observed just one year apart, 5 months and 20 days, and 6 months and 19 days, respectively, had elapsed. In the second of these, however, a dilatation of less than one mm. was found at the expiration of 2 months.

2 (1066)

## A comparison of the effects of glucose and of meat administration upon the non-protein blood nitrogen and the duration of life in experimental renal insufficiency.

By J. H. AUSTIN and S. S. LEOPOLD.

[*From the John Herr Musser Department of Research Medicine and the William Pepper Clinical Laboratory.*]

The following study was undertaken to determine (1) whether those dietary factors that tend to increase the non-protein blood nitrogen in acute insufficiency also tend to shorten the duration of life, and (2) the value of glucose in prolonging life in acute renal insufficiency. The method adopted was to observe the daily curve of non-protein blood nitrogen and the duration of life after complete renal insufficiency had been induced by bilateral ureteral ligation in a series of dogs; half the animals being given glucose, the other half, meat.

Six dogs were divided into two groups of three each. One group was fed upon beef heart, the other upon glucose dissolved in water. On the third day, 5 c.c. of blood was taken from the jugular vein for estimation of the total non-protein nitrogen by the Folin method, and immediately thereafter each dog was etherized and both ureters ligated. On the following day, blood

was again taken in the same manner and the diet resumed as before operation. Every day thereafter blood was taken before feeding. When the stomach became unretentive, the glucose dogs were given ten per cent. solution of dextrose in distilled water intraperitoneally. The meat dogs when they refused the meat were allowed water as desired.

At autopsy, all the ureters were found satisfactorily ligated, the pelvis of the kidney was filled but not materially distended and there was no evidence of infection. In the animals receiving glucose solution intraperitoneally, there was no evidence grossly of free fluid in the peritoneal cavity.

The following facts were noted. After forty-eight hours on the diets, but before ureteral ligation, the blood nitrogen of the two groups was the same. The day after operation, neither meat nor glucose having been given the day of the operation, the blood nitrogen in both groups was almost the same, possibly a little lower in the glucose group; the loss of weight at this time was the same in the two groups. Forty-five hours after operation, the meat animals having retained their feeding the day previous, while the glucose animals had vomited, the loss of weight was distinctly greater in the glucose group; the blood nitrogen was distinctly lower in the glucose group. Sixty-nine hours after operation only one of the meat group was surviving. All of the glucose group were surviving, and exhibited blood nitrogens comparable with those of the meat group of twenty-four hours earlier. Duration after operation was practically the same in animals 39 (meat) and 34 (glucose); the two other meat animals survived a distinctly shorter time than the two other glucose animals.

In these experiments, the condition that makes for a lower blood nitrogen also appears to make for a longer duration of life after acute renal insufficiency, but for confirmation of these findings and for further investigation of the factors involved, additional studies are in progress.

3 (1067)

## The significance of the uric acid, urea and creatinine of the blood in early and late nephritis.

By V. C. MYERS, M. S. FINE and W. G. LOUGH.

[*From the Laboratory of Pathological Chemistry and the Department of Medicine, New York Post-Graduate Medical School and Hospital.*]

Typical cases of gout show, as a rule, blood uric acid values from 2 to 5 times the normal. The amounts of urea and creatinine are normal or in the case of urea, only slightly above normal. Many early cases of nephritis, especially of the interstitial type, give blood pictures which differ little from those of gout. The uric acid findings are quite as high and the urea content varies from only slightly above to more than double the normal amount. The creatinine is only slightly increased. As the condition of cases of this type becomes more severe, the retention of urea increases, until we have high values for urea as well as for uric acid. If improvement takes place the concentration of urea gradually falls until the picture is that of the preceding group. If, on the other hand, the case goes on to a fatal termination, the retention of uric acid and urea is followed by that of creatinine, the concentration of which may reach twenty times the normal. Here the phthalein output is practically zero.

From the foregoing it would appear that as the permeability of the kidney is lowered it becomes evident in the blood, first, by an increase in the uric acid, second, by that of urea and lastly, by that of creatinine. That this should be the case seems quite plausible when we consider the ease of excretion of these constituents, as determined from a comparative nitrogen partition of normal urine and blood. Uric acid nitrogen forms 2 per cent. of the non-protein nitrogen of both urine and blood, urea nitrogen about 85 per cent. in urine but 50 per cent. in blood and creatinine nitrogen 5 per cent. in urine but only 2 per cent. in blood.

4 (1068)

## The movements of the mitral valve flaps studied by a new method.

By **A. L. DEAN, JR.** (by invitation).

[*From the Physiological Laboratory, Cornell University Medical College.*]

The desirability of a more accurate knowledge of the movements of the auriculo-ventricular valves led to the adoption of the following method of study in the perfused cat's heart: The margins of an opening in the left auricle are stitched to the bottom tube of a small cylindrical reservoir in which the height of pressure is regulated and varied by a set of lateral overflow-tubes. To one of the mitral valve flaps is attached a human hair communicating with a short and delicate lever of straw, held upward by slight spring tension. Whenever the valves move up the lever is elevated and a small mirror attached to the axis of the lever system is depressed. Upon this mirror is projected a beam of light and through its reflection the movements of the mirror are recorded upon a moving bromide surface. In this way the oscillations of the valve flaps may be optically recorded and compared with simultaneous optical tracings of auricular and ventricular activity. To prevent the transfer of ventricular movements to the threads connecting with the valves, the heart is fastened by stitches, carefully placed around the auriculo-ventricular ring to a neatly fitted ring of metal.

A study of the records thus far obtained indicates that the following movements of the auriculo-ventricular valves occur in every cardiac cycle: A very short interval after the *onset of auricular systole*, the cusps are slightly depressed toward the ventricle, but before the *end of auricular systole* they quickly ascend toward the auricle. With the onset of *auricular diastole* (which in these experiments began a distinct interval before subsequent ventricular systole) the valves move ventricle-ward to their former position. With the onset of *ventricular systole* the cusps immediately move upward and close completely. So they remain until ventricular relaxation begins. During *ventricular diastole* the valves move ventricle-ward to a position that is lower

than that occupied previous to either auricular or ventricular systole. In the latter portion of diastole (diastasis) they gradually float upward but to a slight extent only. Superimposed upon the main curves of closure are found oscillations of much smaller amplitude and of shorter period. Further investigation is necessary before it may be justly concluded that these correspond to the vibrations responsible for the heart sounds.

The conclusion is reached that the mitral valves undergo two movements toward closure in each cardiac cycle, the first near the end of auricular systole, which is transient and incomplete, and the second lasting throughout ventricular systole which is complete and insures the effective closure of the valves.

5 (1069)

## Blood fat in relation to heat production and depth of narcosis.

By J. R. MURLIN and J. A. RICHE.

[*From the Physiological Laboratory of the Cornell University Medical College, New York City.*]

Experiments on dogs have been designed to answer the questions: (1) whether fat injected directly into the circulation can be oxidized at once, and (2) what is the relation between the concentration of fat in the blood and the heat production. Incidentally it has been necessary to determine whether the depth of narcosis had any effect on the amount of fat in circulation, and on the heat production.

The following experiments may be cited as typical of the effect on percentage of blood fat and on heat production, of a single intravenous injection of 100 c.c. 3 per cent. emulsion of lard oil. It will be seen that the heat production rises and the R. Q. falls as the fat becomes more concentrated in the blood, indicating, therefore, that the injected fat burns.

In order to insure complete muscular rest chloretone was administered to a number of the animals and it was while controlling the effect of this narcotic that the following observations were made: (1) The percentage of blood fat runs parallel with the depth of narcosis, *i. e.*, the deeper the narcosis the lower the blood

| Dog No. | Wt. Kgm. | Hour. | Liters $CO_2$ per Hr. | Liters $O_2$ per Hr. | R. Q. | Per Cent. Blood Fat (Carotid). | Heat Production Cal. per Hour. |
|---|---|---|---|---|---|---|---|
| 102 | 7.0 | I | 2.171 | 3.013 | .72 | 0.45 | 14.17 |
| | | II | 2.478 | 3.108 | .79 | 0.47 | 14.92 |
| | 100 c.c. | emulsion (3 per cent.) by jugular vein | | | | | |
| | | III | 2.327 | 3.100 | .75 | 0.51 | 14.69 |
| | | IV | 2.627 | 3.653 | .72 | 0.58 | 17.18 |
| 111 | 7.0 | I | 2.581 | 3.016 | .85 | | 14.66 |
| | | II | 2.578 | 3.023 | .85 | | 14.70 |
| | 100 c.c. | emulsion (3 per cent.) by jugular vein | | | | | |
| | | III | 2.435 | 3.008 | .81 | | 14.48 |
| | | IV | 2.442 | 3.251 | .75 | | 15.41 |
| | | V | 2.494 | 3.412 | .73 | | 16.09 |

fat; (2) following a single injection (intraperitoneal, in mineral oil) the heat production rises as the narcosis wears off, independently of muscular motions, but parallel to the percentage of fat in the blood. The following experiment illustrates the point.

| Dog No. | Wt. Kgm. | Hour. | Liters $CO_2$ per Hr. | Liters $O_2$ per Hr. | R. Q. | Per Cent. Blood Fat (Carotid). | Heat Production Calories per Hr. |
|---|---|---|---|---|---|---|---|
| | | | | | | 0.41 | |
| 117 | 9.0 | I | 1.880 | 2.223 | .85 | | 10.81 |
| | | II | 1.812 | 2.038 | .89 | | 10.01 |
| | | | | | | 0.44 | |
| | | III & IV[1] | 2.08<br>2.08 | 2.401<br>2.401 | .87 | | 11.74<br>11.74 |
| | | | | | | 0.46 | |
| | | V | 2.093 | 2.458 | .85 | | 11.95 |
| | | VI | 2.081 | 2.589 | .80 | | 12.43 |
| | | | | | | 0.51 | |

This relationship as regards depth of narcosis and percentage of fat in the blood has been confirmed with morphine. With ether a second narcosis had the effect of raising the blood fat to a higher point than the first.

Blood fat has been determined by the nephelometric method and heat production by indirect calorimetry.

[1] A single two-hour period.

6 (1070)

## The interpretation of a positive nitrogen balance in nephritis.

By HERMAN O. MOSENTHAL, M.D.

[*From the Medical Clinic of the Johns Hopkins Hospital, Baltimore.*]

In studying nitrogen metabolism in certain cases of nephritis, a retention of this substance was observed. The conception of the retention of nitrogen in nephritis, as understood by the clinician, generally implies two facts: Firstly, that a positive nitrogen balance is usually due to kidney insufficiency; secondly, that the retained nitrogen is present in the body as waste-nitrogen and circulates in the blood, in part, at least, as non-protein nitrogen. It is known from the work of Marshall and Davis[1] that urea is evenly distributed throughout the body, except in certain tissues, as the fat, bone, cartilage, etc., which do not take up urea. In calculating the theoretical amounts of non-protein nitrogen to be expected in the blood, it has been assumed that all the nitrogen which the body has metabolized and is about to excrete, in

TABLE 1.

THEORETICAL AND ACTUAL VALUES OF NON-PROTEIN NITROGEN OF THE BLOOD RESULTING FROM NITROGEN RETENTION IN CERTAIN CASES OF NEPHRITIS.

| | N. of Blood—Mg. per 100 c.c. | | | N. Grams Retained During Observation. |
|---|---|---|---|---|
| | At Beginning of Observation. | At End of Observation. | Theoretical Value at End of Observation.[2] | |
| Case 1 | 30 | 37 | 152 | 92.0 |
| " 2 | 30 | 38 | 116 | 65.0 |
| " 3 | 25 | 34 | 93 | 51.0 |
| " 4 | 30 | 37 | 165 | 101.4 |
| " 5 | 29 | 27 | 119 | 69.1 |
| " 6 | 71 | 74 | 117 | 35.3 |

contradistinction to the nitrogen which the tissues are storing is evenly distributed throughout the body as is the case with urea.

[1] Marshall and Davis: *Jour. Biol. Chem.*, 1914, XVIII, 53.

[2] These figures represent the values obtained for non-protein nitrogen of the blood at the beginning of the observation plus the theoretical value due to retained nitrogen.

Applying these principles to the total non-protein nitrogenous products, it is found that in a subject of average weight, for every gram of nitrogen retained, the non-protein nitrogen of the blood should be increased 1.33 mg. per 100 c.c. According to these calculations, in the cases presented here, if none of the retained nitrogen were assimilated or stored, and all of it circulated as waste-nitrogen because the kidneys did not excrete it, the figures shown in the table would be obtained.

This table shows that a positive balance of nitrogen in cases of nephritis on a mixed diet is not necessarily followed by a corresponding increase in the non-protein nitrogen of the blood. It is evident that discretion must be exercised in interpreting a normal figure for non-protein nitrogen of the blood as indicating that no nitrogen retention has taken place, and in considering a positive nitrogen balance as an absolute indication of the inability of the kidney to excrete this substance.

7 (1071)

## On the occurrence and distribution of potassium in normal and nephropathic kidney cells.[1]

By WM. DEB. MAC NIDER.

[*From the Laboratory of Pharmacology, The University of North Carolina.*]

The observations which are contained in this summary are based on the microchemical demonstration of potassium in the kidney cells of thirty-four dogs. The animals have varied in age from four months to something over ten years. Four of the animals may be grouped as "normal animals." They did not receive any nephrotoxic substance and neither were they subjected to the action of an anesthetic. After a period of three days of observation these animals were killed by shooting.

The remaining thirty animals were rendered nephropathic by uranium nitrate in the dose of 4 mg. or 6.7 mg. per kilogram. They were anesthetized by either Gréhant's anesthetic in 60 per cent. strength, or by morphine-ether.

[1] Aided by a grant from the fund for scientific research of the American Medical Association.

At the termination of the experiment small pieces of kidney tissue were removed, and frozen sections not over 20 micra in thickness were made. The sections were treated at once with Erdmann's[1] reagent as modified by Macallum[2] and used by him in his studies "On the Distribution of Potassium in Animal and Vegetable Cells."

The reagent which consists in a solution of the hexanitrite of cobalt and sodium serves as a complete precipitant of potassium from its solutions, in the form of an orange-yellow precipitate of the triple salt. If the salt is present in minute quantities the crystalline form is absent. To render the detection of small quantities of the salt possible, Macallum[3] used ammonium sulphide to react with the cobalt of the salt and form the black sulphide of cobalt which is easily detected. This suggestion of Macallum's has been employed in the demonstration of potassium in all of the sections.

The results which have been obtained are as follows.

1. The epithelial cells of the normal dog kidney show only traces of potassium. The potassium is most marked in the loops of Henle and is fairly evenly distributed throughout the cytoplasm of the cells. It has never been demonstrated within the nucleus of the normal cell.

2. The epithelium of the nephropathic kidney shows an increase in potassium over that of the normal. The potassium differs in distribution within the cytoplasm of the cell and has been demonstrated within the nucleus of the cell.

3. The potassium in the nephropathic organs has been especially marked in the cells of the convoluted tubules. In the cytoplasm of the cells forming these tubules the potassium is not uniformly distributed but is found to collect along the free margin of the cells bordering the lumen of the tubule. A similar observation on the distribution of potassium salts was first made by Macallum[4] in his studies of the frog kidney in which a decinormal solution of potassium chloride was injected into the dorsal lymph sacs of frogs.

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[1] "Anorganische Chemie," 1898, p. 630. Reference given by Macallum.

[2] *Jour. Phys.*, Vol. XXXII, No. 2, p. 98.

[3] *Loc. cit.*

[4] *Science*, Vol. XXXII, No. 824, p. 497.

4. Such accumulations of potassium salts are as marked in the kidney epithelium of nephropathic animals which are polyuric, as they are in the nephropathic animals which have been rendered anuric.

5. The above observation would tend to minimize the importance of potassium in being responsible for a lack of function on the part of the kidney.

6. The age of the animal has apparently no constant influence on the amount of potassium microchemically demonstrable. However, the oldest animal of this series showed the most marked potassium precipitate. In this animal, and one other of the series, which were anuric from uranium, and in which the epithelium of the convoluted tubules had undergone a severe swelling and partial necrosis, not only did the cytoplasm of these cells give the potassium reaction but potassium was also demonstrated in the nucleus of the cell.

8 (1072)

## The action of animal extracts upon the flow of bile.

By ISAAC OTT, M.D., and JOHN C. SCOTT, M.D.

[*From the Laboratory of Experimental Research, Medico-Chirurgical College of Philadelphia.*]

Our experiments were made upon etherized cats. We injected secretin at intervals and determined that equal doses of secretin were followed by equal increments in the bile secretion. The cystic duct was previously ligated close to the common duct into which a glass cannula was inserted. After determining the effect of a dose of secretin, we waited some time and then injected an equal dose of secretin plus the infusion of the animal extract. The drops of bile were counted for five-minute periods. We found that adrenalin and the hypophysin of Führer (pituitrin) greatly slowed the secretion. Pancreas slightly diminished the secretion. Thyroid extract had hardly any effect. Tonsil extract caused a marked increase. Thymic extract decreased it. Parathyroid, mammary and corpus luteum had no action.

9 (1073)

## Gastro-intestinal studies XI. Studies on the relative digestibility and utilization by the human body of lard and hydrogenated vegetable oil.

By C. A. SMITH, RAYMOND J. MILLER and PHILIP B. HAWK.

[*From the Department of Physiological Chemistry, Jefferson Medical College, Phila.*]

Two normal men were the subjects of the experiment, which was conducted in two periods of eight days each, separated by an interval of three days. The diets were so arranged that the fat, ingested during the first period, was mostly lard, while that of the second period was mostly hydrogenated vegetable oil. The daily feces were analyzed for total fat, fatty acid, and neutral fat by the Saxon method. The average percentage of digestion of lard was 96.75, and of the hydrogenated vegetable oil, 96.3, while the average utilization percentages were 94.7 and 93.35 respectively. It is thus apparent that the hydrogenated vegetable oil used in this experiment was as satisfactorily digested and utilized by normal men as was lard.

10 (1074)

## The ammonia of the gastric juice. (Preliminary communication.)

By HARRY L. HUBER. (by invitation).

[*From the Hull Biological Laboratories of the University of Chicago.*]

Recently Carlson[1] reported some observations on the occurrence of $NH_3$ in the gastric juice of man and of dogs. Further observations have been made and a few of the results are given below.

Three series of experiments were conducted: (*a*) On dogs with Pawlow stomachs; (*b*) on normal human individuals, and (*c*) on human individuals with gastric disturbances.

The $NH_3$-content of the gastric juice of normal dogs varied

[1] Carlson, *Am. Journal of Physiology*, 1915, XXXVIII, p. 248.

in different dogs between 0.5–3.5 mg. $NH_3$ per 100 c.c. of juice, there being some variation from day to day, and at different times during the same day in the same dog. The juice was collected for a period before feeding and one-hour periods after feeding. The addition of $NH_4Cl$ to the dogs' food caused in each instance an increase in the $NH_3$-content of the gastric juice collected from the Pawlow pouch. Three dogs, in which gastric ulcers had been produced experimentally showed an increase in the $NH_3$-content at irregular intervals. After a time two of these dogs refused to eat and the juice collected during this time showed a progressively increasing content of $NH_3$. At autopsy these dogs showed either active or healed ulcers, usually located in the Pawlow pouch.

*b.* The experiments on normal healthy individuals were conducted on men who were connected with the laboratory. The juice was collected by means of the Rhefus stomach tube before and after feeding on Ewald meal. With three exceptions the $NH_3$-content of the juices from these men ranged between 0.5–3.5 mg. of $NH_3$ per 100 c.c. of juice. In these three men the $NH_3$-content ranged between 10–15 mg. $NH_3$ per 100 c.c. of juice.

A series of experiments was then conducted on one of these men with high $NH_3$-content. The diet was so arranged that there were periods of low protein and of high protein ingestion, and the $NH_3$ content of the gastric juice and the total $NH_3$ of each day's urine were noted. During low-protein ingestion the $NH_3$ content of the gastric juice fell in five days from 12–3.5 mg. and the $NH_3$ of the urine also showed a marked decrease. During the high-protein ingestion the $NH_3$-content of the gastric juice rose in 3 days from 12–28 mg. $NH_3$ and the $NH_3$ of the urine also showed a marked increase. In both cases the $NH_3$ in the gastric juice and in the urine came back to the original level within two days after resuming the usual diet.

In the second experiment the diet was kept uniform throughout the period and during certain periods an excess of alkalis or of acids was added. During the period of excess alkali ingestion the $NH_3$ of the gastric juice remained the same while the total $NH_3$ of the urine fell to 1/10 its former level. During the ingestion of acid the $NH_3$ of the gastric juice again remained the same while

the total $NH_3$ of the urine was increased. When the alkali and the acid were left out of the diet the normal level for the $NH_3$ of the urine was reached in a short time.

*c.* Estimation of the $NH_3$-content of Ewald meal juice from 26 individuals with gastric disturbances, supposedly ulcers, was made and in only five of these was the NH-content markedly increased. In two of these cases the diagnosis of carcinoma of the stomach was made with certainty; in another case a diagnosis of ulcer with obstruction was made and in the remaining 2 cases a diagnosis of gastric ulcer was made. Further work is being conducted along this line with the view of determining the source and the significance of the gastric juice ammonia.

11 (1075)

## The action of heavy metals on the isolated intestine.

By WILLIAM SALANT and C. W. MITCHELL.

[*From the Pharmacological Laboratory of the Bureau of Chemistry, Washington, D. C.*]

In experiments with zinc which was used in the form of the malate and carried out on isolated segments of the intestines of cats and rabbits by the method of Magnus it was found that even low concentrations may produce depression of muscular activity. A solution *N*/20,000 zinc malate, proved to be quite active in some experiments. *N*/10,000 and *N*/5,000 zinc malate produced, after a brief preliminary stimulation, considerable decrease and sometimes irregularity of the force of rhythmic contractions. Occasionally decrease of frequency and tonus were also observed.

When the segments of the intestine were suspended in pure Locke solution again, some improvement occurred, although it had been acted upon by zinc 45–70 minutes. In experiments with concentrations of *N*/1,000 and *N*/500 and sometimes even with *N*/2,000 permanent injury to the tissues may be caused by the metal as no recovery could be observed when pure Locke solution was substituted for one containing zinc.

The action of nickel employed in the form of the acetate was also tested. Dilute solutions, *N*/10,000 and *N*/5,000 produced

temporary depression followed by recovery and sometimes stimulation while the intestine was still in the solution of the salt. Complete abolition of rhythmic contractions and decrease of tonus were observed when much higher concentrations were used, but the effect was not permanent in these experiments as recovery in pure Locke solution took place after the intestine had been suspended in *N*/500 nickel acetate for twelve minutes. The reaction to pilocarpine and of barium was studied in experiments with both of the metals. The evidence obtained points to injury to nerve endings as well as of the muscle fiber, but the latter was in some experiments much more resistant.

12 (1076)

## A note on the failure of pituitrin to sensitize the sympathetic system.

By **R. G. HOSKINS** (by invitation).

[*From the Laboratory of Physiology of the Northwestern University Medical School.*]

In 1912 Kepinow published the conclusion that the injection of small quantities of pituitary extract "sensitizes" the point of attack of epinephrin.[1] His observations were that a given dose of epinephrin produced a greater mydriatic effect in rabbits and cats, a greater vasoconstriction in the Loewi-Trendelenberg frog preparation and a greater vasomotor effect in rabbits and dogs if immediately before hand a minimal dose of pituitary preparation had been injected. Kepinow's work has been quoted as the basis for a rather far reaching conclusion that the pituitary gland has normally the function of promoting the activity of the sympathetic nervous system.

In various researches on the vasomotor system we have had occasion to use pituitary extract in connection with epinephrin and nicotin and it became important to know to what extent sympathetic sensitization occurs. As our previous work has been done exclusively on dogs we have investigated the matter in this

[1] Kepinow, *Archiv für experimentelle Pathologie und Pharmakologie*, 1912, LXVII, 247.

species only. Kepinow used two kinds of pituitary material, an extract prepared by himself, and the commercial preparation "Pituitrin" made by Parke Davis and Co. Kepinow states that both preparations gave similar effects. Our experiments were made with "Pituitrin" only.

Since the results are negative they may be reported very briefly. The procedure was to determine the effects of injections of given quantities of "adrenalin" and of nicotin, selected to give a moderate rise of blood pressure. About 1 c.c. of adrenalin, 1 : 100,000, and 1 c.c. of nicotin 1 : 4,000 are suitable for medium-sized dogs. Having determined the reactions to these drugs, pituitrin was injected by vein in quantity to give a slight rise of pressure, *e. g.*, .05 c.c. At various intervals from one half to several minutes after this injection, the adrenalin and nicotin injections were repeated. In no case was a significant change of reaction noted. In most of the animals the vagi were cut, but this procedure made no apparent difference in results.

In view of the restricted value of negative results some hesitancy is felt in offering them for publication. In further consideration, however, of the vast number of unjustified generalizations in the literature of internal secretion they are offered for what they may be worth.

13 (1077)

## The production of atrioventricular rhythm in man after the administration of atropin. (Preliminary Communication.)

By **FRANK N. WILSON, M.D.** (by invitation).

[*From the Department of Internal Medicine, University of Michigan.*]

During the past year two patients with cardiac complaints were given repeated injections of 1 mg. atropin sulphate and on each occasion atrioventricular rhythm was observed. This usually appeared in from eight to ten minutes after the drug was given, it persisted for only a few minutes, and disappeared before the maximum effect of the drug was reached.

In order to determine whether this tendency to A–V rhythm was peculiar to these patients or whether it exists normally, a

series of experiments on normal individuals was carried out. Eighteen subjects, all under twenty-eight years of age and all with apparently normal hearts, were given hypodermic injections of 1 mg. of atropin. The effect of deep breathing and of ocular pressure upon the cardiac mechanism was studied both before and during the atropin action. Before the injection, A–V rhythm was not produced in any of the subjects tested. Between eight and fifteen minutes after the injection, however, A–V rhythm could be produced by ocular pressure or deep respiration in the majority of the subjects. After the atropin effect had reached its height A–V rhythm could no longer be produced.

Three types of A–V rhythm were observed. In the first, which occurred most frequently, the P–R interval was reduced and P was inverted; in the second, the P–R interval was zero; while in the third there was an R–P interval. The last was observed in only two subjects. These differences evidently depended upon the level of the pacemaker in the junctional tissues.

These observations may be explained on the assumption that atropin releases the A–V tissues from vagus control somewhat before it releases the sinus node. At this time stimulation of the vagus slows the sinus rhythm without a correspondingly great effect upon the inherent rhythms of the lower centers. The latter therefore tend to usurp the pacemaking functions of the heart. After full atropin action on the other hand, both the sinus and the lower centers are released and the more rapid sinus rate controls the cardiac rhythm. Before atropin vagus stimulation probably slows the inherent rhythms of the lower centers as well as that of the sinus so that the former do not ordinarily escape.

14 (1078)

## Studies on so-called protective ferments VIII. On the mechanism of anaphylaxis and antianaphylaxis.

By J. BRONFENBRENNER.

[*From the Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

As we have reported a little over a year ago,[1] the interaction between an immune serum and its corresponding substratum is followed by a formation of toxic split products. We found that the toxic material originated not from the substratum or antigen, but from the serum itself.[2] These findings threw light on some of the unsettled questions in the theory of anaphylaxis and antianaphylaxis. Experiments conducted with the view of correlating our findings with the accepted views on this subject, suggested a following hypothesis about the nature and mechanism of anaphylaxis.

Blood serum contains normal proteolytic ferments which require special conditions of the medium in order to exhibit their activity. Normally the degree of concentration of colloids in the serum offers an obstacle to the activity of these ferments. In the experiments in vitro it is possible to change the degree of concentration of colloids in the serum, thus diminishing its antitryptic inhibiting power and setting free the ferments.[3]

This activation of normal serum can be accomplished by mechanical adsorption, as in experiments of Plant, Peiper and others, or by the dissolution of some of the serum colloids, as in the experiments of Jobling.[4] In either case the degree of dispersion of remaining colloidal particles is increased and thus ferments are allowed to act.

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[1] Bronfenbrenner, *Pennsylvania State Journal*, 1914, October, p. 20.

[2] Bronfenbrenner, PROC. SOC. EXP. BIOL. AND MED., 1914, XII, p. 7–8; also *Journ. Exp. Med.*, 1915, Vol. XXI, No. 5, p. 480.

[3] Bronfenbrenner, *Journ. Exp. Med.*, 1915, XXI, No. 3, p. 221.

[4] Jobling and Peterson, *Journ. Exp. Med.*, 1914, Vol. XIX, p. 239. Though the authors find it necessary to remove the lipoid in order to activate the enzyme, our own experiments show that the removal of lipoid is not necessary. Mere bubbling of ether vapor through the serum accomplishes the activation.

Our experiments have shown that also the physico-chemical changes following the specific interaction between the antigen and antibody influence the colloidal conditions of the medium in the same manner.[1] Our records show that both stalagmometer and refractometer register the increase of dispersion in the immune serum following the addition of the specific antigen and parallel with it the actual measurements of the antitryptic titer of the serum show a steady diminution of the power of this serum to check the activity of its own proteolytic ferments.[2]

In anaphylaxis the latter process takes place, namely, if a suitable amount of antigen is injected into a sensitized animal, the interaction between the specific antibodies and the antigen produce a physico-chemical change in the serum, followed by a diminution of its antitryptic activity. Once the balance between the tryptic and antitryptic powers of the serum is destroyed, the proteolytic ferments may attack the protein of the serum with the production of toxic split products, and anaphylactic shock follows.[3]

That the mechanism of anaphylaxis rests on the disruption of balance between the tryptic and antitryptic properties of the serum is especially evident from our experiments in which we succeeded in preventing anaphylactic shock in experimental animals by increasing the antitryptic power of their serum at will before subjecting them to shock.[4] In doing so we found that practically any substance which caused the rise in antitryptic titer of the serum of experimental animals, protected them also from the subsequent anaphylactic shock. We found also that all such substances are toxic by themselves if injected in sufficient quantity. The mechanism of this protection seems to be as follows.

The introduction of poisons in quantities not sufficiently large to kill the animal outright is followed by the death of the tissues immediately affected by the poison. With the death of the tissues

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[1] Bronfenbrenner, PROC. SOC. EXP. BIOL. AND MED., 1914, XII, p. 4.

[2] Bronfenbrenner, Mitchell and Titus, *Biochemical Bulletin.*

[3] Bronfenbrenner, *Penna. State Med. Journ.*, October, 1914; also *Journ. Exp. Med.*, 1915, Vol. XXI, p. 480. In a current number of the *Journ. of Exp. Med.*, this view of anaphylaxis is corroborated by Jobling, Peterson and Eggstein.

[4] Bronfenbrenner and Schlesinger.

the intra cellular ferments are set free.[1] These ferments possibly with the collaboration of the ferments thrown out from the surrounding fixed cells as well as from blood serum and leucocytes proceed to dispose of the dead material. Some of these split products of protein constituents of digested tissue cells, together with some non-protein constituents (lipoids?) of these cells, exert antagonistic antitryptic action, and retard or stop the activity of proteolytic ferments.

Since, as it was suggested before by us,[2] the specific anaphylactic shock is due to the intoxication of the animal following the liberation of proteolytic enzyme in its blood, it is possible that the preliminary injection of a suitable amount of poison causes the increase of the amount of protein split products in the circulation of the animal and the resulting change in the degree of colloidal dispersion paralyzes the activity of proteolytic ferments which are liberated upon the subsequent introduction of a lethal dose of antigen into a sensitized animal.

The effect of the vaccinating injection of a sublethal dose of antigen into sensitized animals, or of the vaccinating injection of a sublethal dose of anaphylatoxin into normal animals, is evidently due to the same mechanism of partial proteolysis followed by the output of split products acting as antitrypsin and not to the exhaustion of antibody.

Usually the anaphylactic state is taken to be the opposite to the state of immunity. The above theory makes both the active immunity and anaphylaxis a part of the same process. The difference between the two reactions being only in the rapidity and extent of proteolysis induced by the specific combination of antigen with its antibody in vivo.

[1] In the experiments which are to follow we will show the actual changes in the blood and urine following the liberation of ferments during the specific anaphylactic shock, as well as during nonspecific proteolysis due to poisoning.

[2] Bronfenbrenner, *Bioch. Bull.*, March, 1915, p. 87.

15 (1079)

## Icterus. A rapid change of hemoglobin to bile pigment in the pleural and peritoneal cavities.

By C. W. HOOPER, M.D., and G. H. WHIPPLE, M.D.

[*From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.*]

In an earlier communication we have been able to show that bile pigment could be formed from hemoglobin without the agency of the liver. Solutions of hemoglobin were introduced into the blood vessels of dogs whose livers had been excluded from any part in this reaction. There was a prompt formation of bile pigment from hemoglobin with no possible direct liver action. This transformation can take place within a space of two hours when active circulation is maintained in the head and thorax alone. It seemed probable that the endothelium might be the tissue whose activity was responsible for this change of hemoglobin to bile pigments. This work has received confirmation from experiments of McNee.

All our experiments were performed on normal dogs. Hemoglobin in crystalline form dissolved in salt solution or obtained from freshly laked red blood corpuscles was introduced into the pleural or peritoneal cavities. The fluid was withdrawn after different intervals varying from eight hours to three days. Careful tests showed at times some bile pigment formation in eight hours but always in twenty-four hours—often in sufficient amounts to be estimated quantitatively. The amount of bile pigment formation is considerable after an interval of two, three, or four days—even more than five milligrams in some cases. It is to be recalled that a dog of thirty pounds in weight may excrete normally about 25 milligrams of bile pigment in six hours.

There is very good evidence that bile pigments may be formed from hemoglobin by the agency of endothelial cells. There is conclusive evidence that bile pigments can be formed by the mesothelium of the serous cavities. It is possible that this capacity of transforming hemoglobin into bile pigments may be a general property of living protoplasm.

## TABLE I.

### HEMOGLOBIN CHANGED TO BILE PIGMENT IN THE PLEURAL CAVITY.

| Dog No., Date. | Weight, Pounds. | Hours in Cavity. | Bile Pigment Tests. | Bile Pigments in Milligrams. | Fluid Introduced in cc. | Fluid Recovered in cc. | Bile in Urine: Before. | Bile in Urine: After. | Remarks. |
|---|---|---|---|---|---|---|---|---|---|
| 15–11, March 17... | 14 | 8 | 0 | 0 | 620 | 100 | 0 | 0 | Fresh dog hemoglobin. |
| 15–11, March 17... | 14 | 24 | + | — | 620 | 153 | 0 | 0 | Fresh dog hemoglobin. |
| 15–34, May 12 .. | 55 | 8 | (?)+ | — | 1,580 | 1,175 | 0 | 0 | Fresh dog hemoglobin. |
| 15–39, May 10... | 32 | 7 | (?)+ | — | 1,160 | 930 | 0 | 0 | Fresh dog hemoglobin. |
| 15–40, May 10... | 22 | 6 | (?) | — | 700 | 555 | 0 | 0 | Fresh dog hemoglobin. |
| 15–41, May 12... | 36 | 8 | (?)+ | — | 1,480 | 1,230 | 0 | 0 | Fresh dog hemoglobin. |
| 15–39, May 17... | 32 | 17 | +++ | 0.14 | 1,105 | 390 | 0 | 0 | Fresh dog hemoglobin. |
| 15–40, May 17 .. | 21.5 | 18 | +++ | 0.09 | 805 | 265 | 0 | 0 | Fresh dog hemoglobin. |
| 15–52, June 16... | 41.5 | 24 | ++++ | 0.18 | 1,000 | 575 | 0 | 0 | Crystalline dog hemoglobin. |
| 15–54, June 16... | 36.5 | 24 | ++ | — | 800 | 395 | 0 | 0 | Crystalline dog hemoglobin. |
| 15–53, June 16... | 29.5 | 25 | + | — | 800 | 408 | 0 | 0 | Crystalline dog hemoglobin. |
| 15–56, June 16... | 39 | 25 | ++ | — | 900 | 318 | 0 | 0 | Crystalline dog hemoglobin. |
| 15–41, May 20... | 36.5 | 43 | +++ | 0.15 | 805 | 254 | 0 | +Faint | Fresh dog hemoglobin. |
| 15–43, May 20... | 34 | 44 | ++++ | 0.36 | 1,100 | 808 | 0 | 0 | Crystalline dog hemoglobin. |
| 15–40, May 25... | 22 | 65 | ++++ | 3.60 | 940 | 430 | 0 | +Faint | Fresh dog hemoglobin. |
| 15–39, May 25... | 32 | 66 | ++++ | 5.40 | 1,100 | 510 | 0 | +Faint | Crystalline dog hemoglobin. |

### HEMOGLOBIN CHANGED TO BILE PIGMENT IN PERITONEAL CAVITY.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 15–20, March 29... | 19.5 | 8 | (?) | — | 985 | 300 | 0 | (?) | Fresh dog hemoglobin. |
| 15–34, May 20... | 56 | 44 | ++++ | 0.25 | 4,000 | 1,360 | 0 | ?+ | Crystalline dog hemoglobin—7 grams. |
| 15–43, June 17... | 33.5 | 28 | + | — | 2,800 | 755 | 0 | + | Crystalline dog hemoglobin—7 grams. |
| 15–41, June 17... | 36.5 | 48 | ++++ | 0.18 | 3,000 | 310 | 0 | + | Crystalline dog hemoglobin—7 grams. |
| 15–57, June 16... | 24.5 | 48 | ++++ | 0.32 | 2,500 | 255 | 0 | + | Crystalline dog hemoglobin—7 grams. |
| 15–55, June 16... | 40.5 | 48 | ++++ | 0.27 | 3,000 | 310 | 0 | 0 | Crystalline dog hemoglobin—7 grams. |

16 (1080)

## On a colorimetric method of adjusting bacteriological culture media to any optimum hydrogen ion concentration.

By S. H. HURWITZ, K. F. MEYER and Z. OSTENBERG.

[*From the George Williams Hooper Foundation for Medical Research, University of California.*]

In most bacteriological laboratories of this country adjustment in the reaction of culture media by titration has largely replaced all other methods. The indicator most commonly employed is phenolphthalein, and the results are expressed in terms of the amount of normal alkali necessary to bring one liter of the medium to the desired reaction (Fuller Scale).

Recent studies have shown that the titrimetric method in its present form is inaccurate. The results of titrations done in this laboratory support the observations of Clark[1] that media titrated to the end point of phenolphthalein and corrected to definite degrees on the Fuller scale have different hydrogen ion concentrations.

An exact knowledge of the reaction of a medium can be gained only from a determination of its hydrogen ion concentration. It is our purpose to present a simple colorimetric method which makes possible the accurate and rapid determination of the hydrogen ion concentration of culture media and their adjustment to any optimum concentration of ionized hydrogen.

For our work we have made use of a set of standard solutions recommended by Levy, Rowntree, and Marriott[2] for determining the hydrogen ion concentration of the blood. These consist of standard phosphate mixtures containing phenolsulphonephthalein. The advantages of this indicator have been set forth by these workers.

The medium is tested first to ascertain what its ionization is before adjustment. This preliminary test can be carried out quickly: to 3 c.c. of fluid is added 0.3 c.c. of a 0.01 per cent.

[1] Clark, W. M., *Jour. Infect. Dis.*, 1915, XVII, 109.

[2] Levy, Rowntree, and Marriott, *Arch. Int. Med.*, 1915, XVI, 389.

solution of phenolsulphonephthalein, the fluid being read directly in the comparator.[1] In most instances the culture fluid has been roughly adjusted by the usual methods so that its reaction falls within the limits of the scale ($pH^{+}$ = 6.4 to $pH^{+}$ = 8.4).

If the medium has not received a preliminary adjustment of reaction, it may be too acid or too alkaline to be read directly. In that event a specimen of the medium is titrated as follows: to a 3 c.c. sample is added *n*/20 acid or alkali solution[2] depending upon the initial reaction of the medium, until a color is obtained which corresponds to the hydrogen ion concentration desired. The conversion of the amounts of *n*/20 solutions read on the pipette into *n*/1 solutions is made by referring to a curve plotted for an average medium in which the amounts of the *n*/20 solutions required are plotted as abscissæ and the corresponding amounts of *n*/1 solutions as ordinates. Final adjustment in reaction must be made with sterile acid or alkali in order to avoid the change in ionization caused by sterilization.

To illustrate the accuracy of the method the results of the titration of five media are given in the table.

TABLE.

| No. of Exp. | Date. | Medium. | Titration by Fuller Scale. | Preliminary Test. | Standard Desired. | *n*/20 Alkali in c.c. | *n*/1 Alkali Added per 25 c.c. | Value of pH Obtained. |
|---|---|---|---|---|---|---|---|---|
| 1 | Aug. 6 | Veal infusion | 1.0 | Below 6.4 | 7.5 | 0.46 | 0.225 | 7.45 |
| 2 | Aug. 7 | Plain broth | 0.8 | 6.9 | 7.6 | 0.17 | 0.073 | 7.55 |
| 3 | Aug. 12 | Liebig's broth | 0.8 | 6.9 | 7.6 | 0.195 | 0.087 | 7.55 |
| | | | | | 7.7 | 0.20 | 0.095 | 7.7 |
| 4 | Aug. 16 | Plain broth | 0.8 | 7.15 | 7.6 | 0.13 | 0.06 | 7.6 |
| | | | | | 7.9 | 0.195 | 0.088 | 7.9 |
| | | | | | | | | 8.15 |
| 5 | | Extract | 1.0 | 6.9 | 7.5 | 0.08 | 0.04 | 7.55 |
| | | | | | 7.9 | 0.21 | 0.096 | 7.9 |

[1] In order to make direct comparisons possible even in the presence of the natural color of the fluid tested, we have constructed a simple device whereby the medium tested serves as a background for the standard test color to which it imparts its own characteristic quality of color.

[2] In order to keep the concentration of indicator during titration the same as its concentration in the standard comparison tubes (0.3 c.c. to 3 c.c. or 1 to 11) the solutions of *n*/20 acid and alkali are so made up that one eleventh of their volume is indicator solution. The solutions are kept in vessels protected against light, air, and moisture, and the apparatus so arranged that the solution can be delivered directly into a graduated one cubic centimeter pipette provided with a ground glass stopcock on the principle of a burette.

The colorimetric method will be found of great value in the adjustment of the hydrogen ion concentration of media for organisms which are sensitive to the reaction of their culture fluids. The method is comparable in a way to the fine adjustment of a microscope. The method of titratable acidity serving only to adjust media coarsely for the growth of the average organism.

17 (1081)

## Sarcoma occurring in a guinea-pig.

By ERNEST C. DICKSON, M.B.

[*From the Division of Medicine of Stanford University Medical School.*

On May 10, 1915, a large male guinea-pig which had seemed to be in good health in the morning became suddenly ill in the afternoon and died within a short time. The animal had been injected some months previously with a culture of what was supposed to be diphtheria bacilli, but it had survived the injection. At autopsy a large freely movable mass was found in the mid-line of the neck on the ventral surface, which was adherent to the underlying tissues about mid-way between the lower jaw and the shoulder girdle. The tumor was apparently encapsulated and showed no attachment to the skin. It measured $3\frac{1}{2} \times 2\frac{1}{2} \times 2\frac{1}{2}$ cm. in the various diameters, was yellowish in color and quite firm. The capsule was fibrous and cut with some difficulty, but the central portion was quite friable. The cut surface was yellow with many mottled patches which were dark red in color.

Surrounding the tumor and in the right axilla were a number of metastatic nodules, the largest of which measured $1\frac{1}{2} \times 1 \times \frac{1}{2}$ cm., and the smallest being about the size of a grain of wheat. Section of the larger nodules showed a cut surface which was identical with that of the large tumor.

The thoracic and abdominal organs showed nothing unusual.

On microscopic examination the body of the tumor is seen to consist of round and ovoid cells which vary considerably in size. In places the cells are closely packed together but in others they are separated by a reticulum of connective tissue. There are

many large areas of necrosis in which practically all cellular structure is lost. There is evidence of an unsuccessful attempt at encapsulation, but the tumor has invaded the connective tissue of the capsule as well as the adjacent areolar tissue.

The majority of the cells of the tumor resemble lymphocytes in size and in appearance. The nuclei are relatively large and are placed excentrically in the cell body. There are a few cells of about the same size in which two nuclei are present, but there are no large, multinuclear giant cells. In addition to the round cells there are other larger cells which vary in size and shape and which contain large, clear vesicular nuclei, and some very large, round cells with small nuclei which are apparently phagocytes.

The stroma of the tumor consists of a reticulum of connective tissue which contains typical spindle-shaped fibroblasts. In some places there is little fibrous tissue, but in others the fibrosis is quite marked. In the portions which have not undergone necrosis there is a rich blood-capillary network in the stroma.

In the necrotic portions of the tumor the cells show varying degrees of disintegration, and the stroma is studded with nuclear debris. In many places the large phagocytic cells are filled with nuclear fragments.

The appearance of the largest of the metastatic nodules is identical with that of the more cellular portions of the tumor, and there is marked necrosis and extensive invasion of the connective tissue of the capsule. The smaller nodules are of much more frequent development and show little evidence of necrosis.

Smears from the tumor and from the metastases showed no eosinophiles and no bacteria. Tissue cultures were not made.

An attempt was made to transplant the tumor into a small number of young guinea-pigs but it was unsuccessful. However as I knew nothing of the tumor until about two hours after the animal had died it was not possible to make the inoculations soon enough to justify any hope that they would be successful.

# SCIENTIFIC PROCEEDINGS.

## Abstracts of Communications.

### Seventieth meeting.

*New York Post-Graduate Medical School, November 17, 1915.*
*President Lusk in the chair.*

18 (1082)

### Production of pneumonic lesions by intrabronchial insufflation of unorganized substances.

By **B. S. Kline** and **S. J. Meltzer.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

Earlier communications from this department have shown that intrabronchial insufflations of virulent pneumococci in dogs produce lobar pneumonia similar in every respect to the lesions of lobar pneumonia as observed in human beings. In later experiments, Wollstein and Meltzer demonstrated that, at least macroscopically, typical pneumonic lesions can be produced also by avirulent pneumococci and by the saprophytic bacillus megatherium. Microscopically it was established that the lesions produced by the virulent pneumococci contained a great amount of fibrin, while the lesions produced by the last-named organisms (avirulent pneumococci and bacillus megatherium) contained only very little fibrin.

In the present series of experiments various unorganized substances were insufflated into the bronchi of dogs which were killed after twenty-four or forty-eight hours. The substances were: aleuronat suspension in starch solution (autoclaved), starch solution, egg yolk, lecithin, egg white and cholesterin. The results were striking and are as follows: aleuronat, starch, egg

yolk and lecithin produced lesions which macroscopically could not be distinguished from those produced by insufflation of pneumococci. Egg white never produced pneumonic lesions of any extent and even in the occasional patches many of the bronchioles and alveoli containing this protein showed microscopically no nucleated cells whatever in their lumina. The few patches produced by cholesterin were associated with the bronchioles and adjoining alveoli. We shall not describe here the nature of the histological pictures of the lesions under discussion; we shall merely mention the fact that those lesions produced by aleuronat and starch were similar microscopically to those produced by virulent pneumococci, that is, the lesion contained in addition to an exudate of similar cells, etc., a good deal of fibrin; while lesions produced by egg yolk and lecithin resembled more those produced by avirulent pneumococci and bacillus megatherium.

In brief, we may say that our experiments have demonstrated definitely that the hepatization of the lungs, similar to the one observed macroscopically in lobar pneumonia, can be produced by such unorganized substances as aleuronat, starch, egg yolk and lecithin, while egg white does not produce such an effect. The following chart illustrates our results:

| Substance Injected Intrabronchially. | No. of Animals. | Lesion. | | Lung Culture. |
|---|---|---|---|---|
| | | Patches of Consolidation. | Lobar Type of Consolidation. | |
| Aleuronat in starch solution | 8 | | + | 8 sterile. |
| Do | 2 | + | | 2 " |
| Starch solution | 7 | | + | 6 sterile. |
| Egg yolk | 11 | | + | 7 "(1?). |
| Do | 1 | + | | " |
| Lecithin | 6 | | + | 4 " |
| Egg white | 5 | − | − | 5 " |
| Do | 4 | + | | 4 " |

19 (1083)

## Demonstration of the appearance after castration of cock-feathering in a hen-feathered cockerel.

By T. H. MORGAN.

[*From the Department of Zoology of Columbia University.*]

In the Seabright race of fowls the male is hen-feathered, *i. e.*, the feathers on the back of the neck (the hackles) and those on the posterior portion of the back (the saddle) are short and less elongated, like those of the female. When the Seabright male or female is crossed to fowls of another race in which the male has the characteristic male-feathering, the $F_1$ males are hen-feathered, or at least the dominance of hen-feathering is more or less complete. In the second generation there are three hen-feathered to one cock-feathered male.

It has been shown by Goodale that removal of the ovary of the hen or of the duck leads to the development of the male-feathering. I tried to discover whether the removal of the testes in the hen-feathered males would cause them to develop the hackles and saddle feathers of ordinary cocks. My first operations were unsuccessful, owing to failure to completely remove the testis. This autumn Dr. H. D. Goodale performed the operation for me on $F_2$ hen-feathered birds that I had reared. At the time of operation some of the saddle feathers were removed. The new feathers that appeared were like those on the ordinary cock bird; not only did they have the characteristic shape but were bright red also. The result leads to the apparently paradoxical conclusion that the removal of the testes of the hen-feathered cock caused him to develop certain characteristic feathers peculiar to the ordinary male.

The most probable interpretation of the effects of removal of the ovary of the hen (an operation that leads her to develop the male plumage) is that the ovary secretes some substance that holds in check the development of the male plumage. Likewise in the hen-feathered male it would seem probable that the testis produces some substance that inhibits the development of the complete male plumage. Possibly this substance is the same as

that produced in the hen that brings about in her the same result, although there is no direct evidence to show that this is the real explanation.

20 (1084)

## Agglutination of bacteria in vivo; its relation to the destruction of bacteria within the infected host and to septicemia.

By C. G. BULL.

[*From the Laboratories of the Rockefeller Institute for Medical Research.*]

An intravenous injection of immune serum causes an abrupt disappearance of the bacteria from the circulating blood of animals having a bacteremia or a septicemia. This is due to an immediate agglutination of the bacteria and to an accumulation of the bacterial clumps in the lungs, liver, spleen, etc. The clumps of bacteria are phagocyted and destroyed mainly by the polymorphonuclear leucocytes which accumulate in the internal organs following an intravenous injection of foreign protein substances. The septicemia or bacteremia does not recur as long as the immune serum is kept in the blood in a sufficient concentration.

These phenomena occur very typically following intravenous administration of specific immune sera in rabbits infected with pneumococci or Shiga dysentery bacilli. If the rabbits are actively immune to these bacteria, the same phenomena follow an intravenous injection of the bacteria. If the immune animals are given sufficient quantities of the bacte ia, death may be caused by intoxication in the absence of a septicemia.

In natural immunity the above described phenomena follow immediately upon an intravenous injection of the bacteria. Rabbits have a comparatively high natural immunity to many bacteria, of which the following have been studied in this respect: typhoid bacilli, colon bacilli, dysentery bacilli of the Flexner group, *Staphylococcus aureus* and *albus*, non-virulent bacilli of the *mucosus capsulatus* group, and non-virulent influenza bacilli. All of these are agglutinated, phagocyted, and destroyed in normal rabbits as pneumococci are in immune rabbits and none of them causes a true septicemia in these animals.

Rabbits exhibit little or no resistance towards a virulent strain of *Bacillus avisepticus* while dogs are not affected by a subtoxic dose. These bacteria are not agglutinated in the circulation of rabbits and soon begin to multiply and produce a fatal septicemia. In dogs, on the other hand, they are agglutinated and rapidly disappear from the circulation and no true septicemia follows, and as stated, a subtoxic dose causes no symptoms in these animals.

Hence, in the several instances studied, agglutination of bacteria within the circulation of the infected animal is followed by an abrupt disappearance of the bacteria from the blood stream, by accumulation of the agglutinated bacteria within the internal organs, and by phagocytosis of the bacterial clumps by the polymorphonuclear leucocytes, and a true septicemia does not arise.

21 (1085)

## Accumulation of nitrogen in the tissues in renal disease.

By HELEN DAVIS and NELLIS B. FOSTER, M.D.

[*From the Department of Medicine, Cornell Medical College, and the New York Hospital.*]

The retention of nitrogen as a manifestation of certain types of renal disease is a well-recognized phenomenon. When the conditions of study are carefully controlled with accurate analyses of the food and excreta the amounts of nitrogen retained in the body is, with severe cases, very large—two grams per day for periods of two weeks is not an exceptional amount. Since these patients are usually quite sick and commonly manifest no sign of improvement so long as the retention persists it is inconceivable that this nitrogen is retained for tissue growth in a physiological sense. On the other hand while the blood of these patients often shows on analysis an increase in non-protein nitrogen the figure may not rise to any significant degree and never becomes sufficiently high so as to account for more than a small fraction of the nitrogen retained. We have also noted with several patients during metabolism studies a flushing out of nitrogen so that there resulted large minus balances during periods of improve-

ment in the patient's condition. It seemed that the explanation of these phenomena is that the tissues withdraw from the blood a large part of the katabolic products which compose the retained nitrogen and that this would be disclosed by analyses of organs and tissues obtained at autopsy.

TISSUE ANALYSES.

| No. | Diagnosis. | Water Content Per Cent. | Total Nitrogen Per Cent. | Extract Nitrogen Per Cent. | Extract Nitrogen, per 100 g. Dry Substance. |
|---|---|---|---|---|---|
| | | *Psoas Muscle.* | | | |
| 4. | Pancreatitis | 72.9 | 2.92 | 0.24 | 0.88 |
| 10. | Peritonitis | 72.8 | 3.06 | 0.23 | 0.84 |
| 11. | Cerebral thrombus | 73.8 | 2.99 | 0.24 | 0.90 |
| 12. | Pneumonia | 77.2 | 3.08 | 0.32 | 0.97 |
| | | *Liver.* | | | |
| 4. | Pancreatitis | 74.4 | 2.52 | 0.18 | 0.70 |
| 10. | Peritonitis | 62.1 | 2.34 | 0.17 | 0.44 |
| 11. | Cerebral thrombus | 73.7 | 3.27 | 0.30 | 1.14 |
| 12. | Pneumonia | 74.8 | 3.35 | 0.26 | 1.03 |
| | | *Psoas Muscle.* | | | |
| 19. | Nephritis with edema | 84.5 | 2.83 | 0.223 | 1.43 |
| 21. | Nephritis with edema | 79.8 | 3.11 | 0.214 | 1.05 |
| | | 82.1 | 2.97 | 0.218 | 1.24 |
| | | *Liver.* | | | |
| 19. | Nephritis with edema | 78.8 | 2.56 | 0.14 | 0.67 |
| 21. | Nephritis with edema | 76.6 | 3.02 | 0.195 | 0.83 |
| | | 77.7 | 2.79 | 0.167 | 0.75 |
| | | *Psoas Muscle.* | | | |
| 3. | Nephritis with N retention | 77.7 | 3.01 | 0.370 | 1.65 |
| 13. | Nephritis with N retention | 78.4 | 3.23 | 0.340 | 1.14 |
| 16. | Nephritis with N retention | 78.8 | 3.04 | 0.383 | 1.80 |
| | | 78.3 | 3.09 | 0.36 | 1.53 |
| | | *Liver.* | | | |
| 3. | Nephritis with retention | 76.5 | 3.10 | 0.36 | 1.53 |
| 13. | Nephritis with retention | 73.6 | 3.32 | 0.20 | 0.75 |
| 16. | Nephritis with retention | 76.7 | 2.95 | 0.30 | 1.28 |
| | | 75.6 | 3.12 | 0.28 | 1.18 |

For over two years we have been making these analyses as opportunity presented. Certain technical deficiencies are met with which make the accuracy of these results only comparative. The chief among these is due to the fact that as soon as tissues such as liver are minced a separation of fluid (blood and lymph) occurs. This is not the case with muscle but is to some degree true with all organs.

We have not utilized materials unless the autopsy occurred soon after death. The following brief table abstracted from a considerable number of analyses is fairly representative.

The results given in the table for cases where no conspicuous renal disease was present are the highest we have noted rather than the average figures. For example, case 11, cerebral thrombosis and arterio-sclerosis, there might here have been a difference of opinion as to whether there was renal disease or not, as evidenced in the sections. With pneumonia the analyses gave usually higher results than in any other disease not associated with frank nephritis.

With cases of nephritis with nitrogen retention there is a notable accumulation of extract nitrogen in both muscle and liver tissue, which, for muscle amounts to over 50 per cent increase above the highest normal. With liver the increase is an average and not invariably to be demonstrated. Nephritis with oedema gives inconstant results although the effort was made to select cases where nitrogen retention could be excluded. This, however, appears difficult since pure chlorid retention in our experience is exceedingly uncommon; there being usually a slight tendency to retain nitrogen which is disclosed only in long continued metabolism experiments.

22 (1086)

## The utilization of " reactor " milk in tuberculo-medicine.

By C. B. Fitzpatrick.

[*From the Bureau of Laboratories, Department of Health, New York City.*]

We cannot in the study of tuberculosis get away from the disconcerting observation that it is the infected individual who is immune

or possessed of increased resistance to tuberculosis. Our previous works[1] on immunity and tuberculosis led me to endeavor to ascertain to what extent milch cows, that gave no clinical signs of tuberculosis, and yet reacted to tuberculin, could be considered immune or possessed of increased resistance. Furthermore, to ascertain if the milk and serum of such "reactors" gave evidence of possessing antibodies or other healing bodies not contained in the ordinary milk and serum of cows not infected with tuberculosis. It is occasionally observed that if a cow which has reacted to tuberculin, be allowed to live, it thrives, apparently even better than some of the non-infected members of the herd. Ten of these "reactors" which were in especially prime condition were carefully selected because they thrived, and gave the physical evidence of having withstood the natural infection, in short because they appeared to be immune or the disease arrested. The milk when injected into guinea-pigs did not produce tuberculosis. The milk was also tested by the Bordet-Gengou phenomenon for tuberculosis and gave negative reactions. The blood-serum of nine were also examined for this reaction; four were definitely negative, four gave a weak reaction, and one a decided reaction but not strong enough for diagnosis. One of these cows went dry and the milk of another was excluded because it readily killed mice in comparatively small doses, when injected subcutaneously. The serum of these cows, when added to glycerin-bouillon cultures of the tubercle bacillus did not inhibit their growth. The ten autopsies on these "reactor" cows showed slight localized lesions in the lungs, and in the bronchial and posterior mediastinal glands in nine cows and in one cow slight generalized lesions were found.

Seven moderately advanced cases of adult pulmonary tuberculosis were fed daily a quart of this "reactor" milk over a period of three months. They gained an average of nine pounds. They increased this average gain during the next two months and ten days to 16 pounds. Six controls, *i. e.*, similar cases living under like conditions were given pasteurized milk, during the same

[1] PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 1910, VII, pp. 77–79; ibid., pp. 104–7; ibid., VIII, pp. 24–28; ibid., pp. 41–43; ibid., Feb. 21, 1912, IX, pp. 49–51; ibid., Feb. 19, 1913, Vol. X, No. 3, pp. 103–107; ibid., Oct. 15, 1913; ibid., June 6, 1914, Vol. XI, No. 6. Collected Studies 1913, Research Lab., Dept. of Health, N. Y. City, N. Y.

period. They lost an average of four pounds. They increased this average loss during the next two months and ten days to six pounds. There was no noticeable alteration in the pulmonary conditions.

All the cases took three pints of milk a day. The special cases, one quart of "reactor" milk and one pint of pasteurized milk. The controls, three pints of pasteurized milk.

The "reactor" milk contained less butter fat than the pasteurized milk. The use of this raw "reactor" milk, judging by its action upon two cases of adult pulmonary tuberculosis, is probably contraindicated in dysentery and hemorrhage. This dysentery case proved fatal. It was used in one far advanced case of adult pulmonary tuberculosis and apparently agreed ith her, although her weight remained unchanged.

We mixed diphtheria antitoxine with milk for the purpose of determining by analogy if the tuberculous antibodies when present in milk, would be destroyed by pasteurization. These mixtures after having been heated were tested with toxin, in order to determine whether any destruction of antibody had taken place. We found that the antitoxine was not materially affected by heating at 60° C. for 20 minutes. Certain milks we have tested showed the presence of some natural substance or antibody which neutralized diphtheria toxine. I wish to thank Mr. E. J. Banzhaf for his aid in making these antitoxine tests.

23 (1087)

## Anaphylatoxin and the mechanism of anaphylaxis.

By RICHARD WEIL.

[*From the Department of Experimental Medicine of Cornell University Medical College.*]

Precipitin is identical with the antibody effective in passive sensitization. This is demonstrated by injecting a guinea-pig with the precipitate formed by a mixture of horse serum with the serum of a rabbit immunized thereto. This guinea-pig, if tested after an interval of three days by the intravenous injection of horse serum, presents a violent, at times a fatal anaphylactic response.

If the precipitating antibody is heated at 72° for one half hour, it loses its precipitating functions, but retains its sensitizing power, though somewhat diminished. Such heated precipitin, sometimes described as "precipitinoid," is known to retain its combining power with antigen. One may conclude, therefore, that the combining power, but not the precipitating power, of antibody is essential in anaphylaxis.

Precipitating antibody heated at 72° for one half hour has lost its capacity to bind complement in the presence of antigen. However, it still retains its sensitizing value. These facts are illustrated by the following experiment.

The serum of a rabbit, immunized to crystallized egg albumen, is diluted with three volumes of salt solution. Part of this diluted serum is heated at 56° for one half hour to destroy complement, and part of it is heated at 72° for one half hour to destroy the precipitating function. In the following table the amounts of diluted serum employed are reduced to correspond to the amount of serum therein contained. The method employed is that of complement fixation.

COMPLEMENT FIXATION TEST.

| | 1 | 2 | 3 | 4 | 5 | 6 | Precipitation. | |
|---|---|---|---|---|---|---|---|---|
| S. 56° | 0.5 | 0.5 | .... | .... | .... | .... | 0.5 | .... |
| S. 72° | .... | .... | 0.5 | 0.5 | .... | .... | .... | 0.5 |
| Salt solution | .... | .... | .... | .... | .... | 0.5 | .... | .... |
| Cryst. egg alb | 0.001 | .... | 0.001 | .... | 0.001 | .... | 0.001 | 0.001 |
| G. P. comp. | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.05 | .... | .... |
| Precipitation | | | | | | | ++ | − |

After incubation for one hour, one c.c. of well-sensitized cells was added to each of the tubes. Hemolysis was complete in tubes 2 to 6 after one half hour, but had not even begun in 1. On the following day tube 1 showed slight hemolysis. The test was made with the usual additional controls. The result shows that the serum heated at 72° does not bind complement. The precipitation reaction shows that it does not precipitate. A series of normal guinea-pigs were injected with graded amounts of the same heated serums used in the above experiment, and were injected two days later with egg albumen to test for passive sensitization. Serum heated at 72° sensitized in amounts of 0.3

c.c. Serum heated at 56° in amounts of 0.2 c.c. The same results were obtained when horse serum and the serum of a rabbit immunized thereto were used as antigen and antibody.

Hence we may conclude that complement plays no role in the anaphylactic reaction. Inasmuch as complement is essential to the production of anaphylatoxin, this is equivalent to saying that anaphylatoxin plays no rôle in anaphylaxis.

24 (1088)

**The isolation of a toxic substance from the blood of uremic patients.**

By **NELLIS B. FOSTER, M.D.**

[*From the Department of Medicine, Cornell Medical College and the New York Hospital.*]

The analyses of bloods from cases of uremia have yielded a substance which is toxic. Control analyses of bloods from a wide variety of conditions not associated with uremia failed to discover a similar substance. Guinea-pigs were used as the test animal and enough material can be recovered from 200 c.c. of uremic blood to cause death. The isolation of the substance was effected by a combination of several methods in current use for the separation of animal bases.

25 (1089)

**The possible association of diabetes mellitus and splenohepatomegaly, Goucher; report of a case.**

By **J. R. WILLIAMS** and **M. DRESBACH.**

[*From the Department of Physiology, Cornell Medical College, Ithaca, N. Y.*]

The following case, which we have recently had under observation, is of clinical and scientific interest because of the evidence it presents of the coexistence of diabetes mellitus and splenohepatomegaly, Goucher.

The history, in brief, is as follows: Patient, male, single;

age 28; civil engineer. Family history negative. Patient had no important antecedent illnesses. Physical examination revealed no associated disease of any organs. Blood was practically normal in the beginning, but showed numerical increase in large lymphocytes and decrease in small lymphocytes as the disease advanced. Polynuclear leucocytes showed no suggestive changes.

Patient died in diabetic coma about two and one half years after illness began. There was nothing unusual in the general course of the disease. It progressed in spite of all efforts to combat it. One thing deserves noting, namely, that fat was metabolized poorly, the acidosis increasing whenever the fat in the diet was increased to offset the loss in body weight.

*Post-mortem Examination.*—The body was embalmed with a fluid containing about 8 per cent. of formalin one hour after death, and the tissues were fixed in formalin, Zenker's fluid and osmic acid twelve hours later.

There was some enlargement of the spleen, its size being $13 \times 10.5 \times 6$ cm. and the weight 338 gm. The enlargement was only slight, therefore. Other organs appeared normal. Microscopically, the kidneys and adrenals were normal. The pancreas showed no general structural change except slight atrophy of the acinal tissue. The islets were normal, though there was some tendency towards hypertrophy. The capillary walls were somewhat thickened. There was no hyaline change in the islet epithelium. The spleen was extensively invaded by large round and oval cells of the endothelial type of Goucher. These cells were especially abundant in the pulp. Mesenteric lymph glands and liver showed the same type of foreign cells, but they were not so numerous as in the spleen. The liver was normal except as mentioned, though there was some vacuolization of the liver cells. The foreign endothelial cells were found only in the blood capillaries of the lobules. Pigmentation of the lymph glands was absent. Unfortunately, we did not examine the bone marrow, as we did not suspect this complication.

We believe that the findings in this case warrant the conclusion that splenohepatomegaly existed. This condition is now thought to be connected in some way with faulty fat metabolism. The etiology, like that of diabetes, is obscure. Whether there was

any causal relation between the two pathological processes exemplified in this instance is a point upon which we have insufficient data for a positive statement.

A more detailed account of the case will be published later.

26 (1090)

## Equilibrium in the precipitation reaction.

By RICHARD WEIL.

[*From the Department of Experimental Medicine of Cornell University Medical College.*]

On the basis of experiments performed with horse serum, and similar antigenic substances, it has long been held that precipitation produced by antigen and antibody never goes on to completion, but that both factors are always present in the supernatant fluid. This has been explained by some as an instance of the law of mass action, by others on the basis of certain analogies of colloidal chemistry.

If a pure substance, such as crystalline egg albumen, separated by Hopkins's method, be used as antigen, the results are quite different. When mixed in proper proportions with its antiserum a precipitate is formed; the supernatant fluid never contains both reactive substances. The results hitherto obtained are due, therefore, to a fallacy of technique, and are traceable to the presence of multiple individual antigens in the antigenic substance employed, with a corresponding multiplicity of antibodies in the antiserum.

27 (1091)

## Equilibrium in the dissociation of precipitates.

By RICHARD WEIL.

[*From the Department of Experimental Medicine of Cornell University Medical College.*]

Chickering found that sodium carbonate extracts of a precipitate, produced by a mixture of pneumococcus substance and its antiserum, contained agglutinating and protective antibody, but

no antigen. If precipitates produced by the combination of horse serum, or egg albumen, and their respective antiserums, be treated with salt solution, or with 1 per cent. sodium carbonate, the resulting extract always contains antigen. It also contains passively sensitizing antibody, but no precipitin.

If such a precipitate be treated with trypsin, or with rabbit's leucocytes, both antigen and precipitin are present in the extract. Sensitizing antibody is also demonstrable.

28 (1092)

## Studies on so-called protective ferments—IX. Antitryptic index in its relation to the clinical manifestations of anaphylaxis.

By J. BRONFENBRENNER.

[*From the Pathological and Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

The convulsions in eclampsia and epilepsy, as well as the respiratory failure in asthma have been repeatedly considered as an expression of anaphylactic reaction, though no definite proofs were offered for such a view. The theory of specific parenteral digestion of Vaughan, as applied by Abderhalden and his pupils to diagnosis of different pathological conditions, brought forward new possibilities of investigation in this direction.

According to results obtained by means of Abderhalden reaction many authors concluded that the patients, suffering from the conditions mentioned above, show active specific parenteral digestion of different tissues, as evidenced by the presence of specific proteolytic ferments in their blood.

As I have shown it elsewhere,[1] this conception of the mechanism of parenteral digestion is not correct. The results obtained by means of the Abderhalden reaction are nevertheless of value as they show t at certain specific changes have taken place in the blood[2] of the patients, by means of which the specific parenteral

[1] Bronfenbrenner, these PROCEEDINGS, 1914, XII, p. 3, and 1915, XII, p. 137; also *Journ. Exp. Med.*, 1915, XXI, p. 221.

[2] According to investigations conducted in this laboratory and confirmed elsewhere, the changes consist in the appearance in the blood of specific antibodies (in this case autolytic in nature) and not specific ferments.

digestion is made possible through the liberation of normal proteolytic enzyme.

We have shown in another paper[3] that the products of such digestion may be toxic and if produced in vivo may cause anaphylactic symptoms. Considering the close outward resemblance between the anaphylactic shock and the symptom complex in epileptic or eclamptic convulsions and asthmatic attack, we were interested to see if these phenomena depend on actual parenteral digestion and production of anaphylatoxin in the same measure as does anaphylactic shock.

We have called the attention to the rôle of antitrypsin in the question of activation of normal protolytic ferments of the blood.[3] We have suggested that the combination of antigen with its antibody is followed by the diminution of antitryptic properties of the blood serum and resulting autodigestion of the serum.[1] We have shown moreover, that the degree of active digestion depends on the amount of antitrypsin present.[4]

Assuming that the phenomena observed in eclampsia, epilepsy, asthma and in similar conditions are due to formation of anaphylatoxin in vivo through the combinations of antigen with the circulating antibody, we attempted to measure the amount of antitrypsin in the blood during and between the acute symptoms of the disease and thus see if there is any connection between the morbid symptoms and parenteral digestion. During the last eighteen months[5] we made a study of a number of cases of epilepsy, eclampsia, asthma, and idiosyncrasy to certain foods, and our findings convinced us of the causative relations existing between the parenteral digestion and the morbid phenomena. We found moreover that this relation can be measured in the values of antitryptic index. We shall publish the protocols of actual experiments in the near future.

[3] Bronfenbrenner, *Penn. State Medical Journ.*, Oct., 1914; also *Journ. Exp. Med.*, 1915, XXI, p. 480.

[4] Bronfenbrenner, these PROCEEDINGS, 1914, XII, p. 6; also Bronfenbrenner, Mitchell and Titus, *Bioch. Bull.*, 1915, No. 13, p. 86.

[5] I wish to express my great indebtedness to Drs. Freeland, Zugsmith, and Titus, of the Western Pennsylvania Hospital, and to Drs. Hutchinson, Barrett, and White, of Dixmont Hospital for Insane, for very courteous collaboration and selection of cases for these studies. Without their valuable collaboration the work would have been utterly impossible.

# SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

**Seventy-first meeting.**

*Rockefeller Institute for Medical Research, December 15, 1915.*

*President Lusk in the chair.*

29 (1093)

## The circulatory reaction to graduated work as a test of the heart's functional capacity.

By THEODORE B. BARRINGER, JR., M.D. (by invitation).

[*From the Medical Service of the House of Relief and the Department of Physiology of Columbia University, New York.*]

As a preliminary to a new form of exercise treatment for cardiac insufficiency which we have described elsewhere, we investigated the test of the heart's functional capacity described by Graüpner. The essential features of his test are the deductions made from the form of the curve of the systolic blood-pressure after measured amounts of work. Although we were unable to confirm his most important results, we believe that the method of making frequent readings of the pulse-rate and systolic pressure after measured amounts of work furnishes the key to this problem of determining the heart's efficiency.

Our work may be divided into two parts. The first consisted in experiments on 23 normal persons. In three persons the graduated work was performed by means of a bicycle ergometer of the type described by Krogh and Lindhard,[1] and in 20 persons by means of dumb-bell and bar movements.

The second part comprised experiments on 32 patients suffering from cardiac insufficiency. The ergometer was used in two patients, and dumb-bell work in the remaining 30.

[1] *Skandinavisches Archiv für Physiologie*, 1913, XXX, p. 378.

### EXPERIMENTS ON NORMAL PEOPLE WITH ERGOMETER.

Each person performed successively increasing amounts of work during which the systolic blood-pressure and pulse-rate increased in direct proportion to the amount of work done. After work the blood-pressure and pulse-rate generally fell rapidly to the original figures. As soon, however, as the work exceeded a certain amount, which varied for different individuals, we regularly found that the systolic blood pressure did not reach its highest point immediately after work, but a minute or so later at a time when the pulse rate had dropped back toward normal. This delayed rise of the systolic blood-pressure after heavy work is of much significance and we direct particular attention to it.

### EXPERIMENTS ON NORMAL PEOPLE WITH DUMB-BELL AND BAR WORK.

Various movements with heavy dumb-bells and a steel bar weighing 25 pounds were carried out on 20 normal persons in a way which permitted an approximate estimation of the foot-pounds of work performed. Naturally the pulse-rate and blood-pressure could not be taken during the work, but they were taken before and every minute or half-minute after work. The delayed rise in systolic blood-pressure was obtained after large amounts of work which varied according to the subject's physique and condition of muscular training.

### EXPERIMENTS ON PATIENTS WITH CARDIAC INSUFFICIENCY USING THE ERGOMETER.

Ten experiments were carried out on two patients who rode the bicycle ergometer for periods of 2½ minutes with successively increasing loads. Seven experiments were made with the same patients turning the bicycle pedals by hand instead of by the feet.

These experiments presented several striking features and some marked contrasts to the experiments on normal persons. A delayed rise in systolic blood-pressure was produced by much less work than in the normal subjects. Again the pressure *during* work, instead of rising decidedly as in normal subjects, rose but slightly or fell below the original level.

The most important result of this particular group of experiments was the discovery that approximately the same amount of work was followed by a delayed rise in systolic pressure whether performed by the legs or arms. This is the first time as far as we can ascertain that this law of circulatory physiology has been demonstrated.

## EXPERIMENTS ON PATIENTS WITH CARDIAC INSUFFICIENCY USING DUMB-BELL WORK.

Several hundred experiments were carried out on thirty different patients. The pulse-rate and blood-pressure could not be taken during the performance of the dumb-bell work but were measured every 30 or 60 seconds after work.

The following example is selected because it represents the usual type of reaction following work.

C. G.—A man aged 31, with a history of 4 attacks of rheumatic fever, and symptoms of cardiac involvement for 4 years. On June 21, the following test was made.

| Time. | Pulse-rate | Systolic Blood-pressure. |
|---|---|---|
| 10:55 | 100 | 136 |
| 10:58 | 88 | 130 |
| 11:00 | 100 | 126 |
| | 250 foot-pounds in 20 seconds (10 lb. bell flexed 10 times) | |
| 11:02 | 108 | 138 |
| :03 | 108 | 136 |
| :04 | 96 | 132 |
| :05 | 92 | 126 |
| :06 | 90 | 128 |
| 11:22 | 96 | 128 |
| | 500 foot-pounds in 35 seconds (10 lb. bell flexed 20 times) | |
| 11:23½ | 106 | 140 |
| 11:24 | 102 | 130 |
| 11:25 | 96 | 136 |
| 11:26 | 92 | 130 |
| 11:28 | 100 | 128 |
| 11:34½ | 96 | 122 |
| | 750 foot-pounds in 45 seconds (10 lb. bell flexed 30 times) | |
| 11:37 | 114 | 146 |
| 11:38 | 102 | 136 |
| 11:39 | 102 | 136 |
| 11:42 | 96 | 136 |
| 11:44 | 100 | 122 |

| | 1000 foot-pounds in 40 seconds (15 lb. bell flexed 27 times) | | |
|---|---|---|---|
| 11:47 | 126 | | 136 |
| 11:48 | 114 | Delayed rise | 142 |
| 11:49 | 108 | | 138 |
| 11:50 | 108 | | 134 |
| 11:51 | 96 | | 138 |
| 11:52 | 96 | | 124 |

Our *clinical experiments* demonstrate conclusively, we believe, that in the pulse-rate and blood-pressure reactions to graduated work we possess a valid test of the heart's functional capacity. If the systolic blood-pressure reaches its greatest height not immediately after work but from 30 to 120 seconds later, or if the pressure immediately after work is lower than the original level, that work, whatever its amount, has overtaxed the heart's functional capacity, and may be taken as an accurate measure of the heart's efficiency.

## METHOD OF PERFORMING OUR TEST OF THE HEART'S FUNCTIONAL CAPACITY.

The apparatus used consists of pairs of 5, 10, 15 and 20 pound dumb-bells, and a steel bar about 40 inches long weighing 25 pounds. Two types of movements are done with the bells.[1] In the first a pair of dumb-bells is held at the shoulder, one in each hand and then pushed alternately above the head and toward the median line until the arms are fully extended. As one bell moves up fairly rapidly the other bell returns to the shoulder, the two moving in a sort of see-saw rhythm. In the other movement a bell is held in each hand, the arms hanging by the side of and close to the body, and then each forearm is alternately flexed, raising the bell to the shoulder. The patient stands or sits according to his condition. But one movement is performed with the steel bar. It is picked up from the floor with both hands raised first to a level with the shoulder then pushed above the head until the arms are fully extended and then quickly lowered to the floor again with a single rapid motion.

It is possible to calculate approximately the number of foot-

[1] These movements were first described to us by Dr. Jacob Teschner, of New York.

pounds of work performed in each of these movements. There is a certain amount of work, however, which we cannot estimate in foot-pounds. When a patient stands holding a pair of dumb-bells at his shoulders, work is done as shown by his circulatory reactions, but we cannot estimate it in foot-pounds. This unknown factor may be ignored, however, for our purpose.

Most adults average 2 feet as the distance through which a bell is pushed from the shoulder to full extension of the arm. In the flexion movement, the distance through which the bell is carried from the side of the body to the shoulder averages from 2 feet to 2 feet 6 inches. Now if a 5 pound bell is pushed through 2 feet, 10 foot-pounds of work are done. If the total number of pushes are 20, 200 foot-pounds are done. For the sake of comparison, the time it takes a patient to do any quantity of work should be noted.

If the patient whose heart is to be tested has but recently recovered from an attack of cardiac insufficiency it is well to start with a pair of five-pound bells, the patient sitting on a stool. Two hundred foot-pounds of work are then given either by flexing or extending the bells alternately. The pulse-rate and blood-pressure are taken every 30 or 60 seconds according to the examples given on a preceding page. After the pressure and pulse have returned to the original level, 300 or 400 foot-pounds are done in the same way. The work is increased with each experiment until we reach a delayed rise in blood-pressure. *The experiment which has caused a delayed rise should always be repeated after a few minutes rest with a slightly increased amount of work for the purpose of confirmation.* When once the amount of work which will produce a distinct delayed rise in blood-pressure is ascertained, it is quite remarkable how slightly the results vary upon a repetition of the experiment with the same or increased work. Yet if our test is valid this should be so.

### THE TESTING OF PATIENTS WHOSE CARDIAC CAPACITY EXCEEDS AN ABILITY TO PERFORM 100 FOOT-POUNDS OF WORK IN 60–90 SECONDS.

People with normal hearts, or patients with well compensated heart lesions, afford a more difficult problem in mechanics when

their hearts are functionally tested. These people are able to flex or extend heavy dumb-bells until their arm muscles are exhausted and yet the heart muscle will show no exhaustion. That is, no delayed rise in the systolic blood-pressure is obtained. Here it is necessary to use more powerful muscles, capable of doing much greater amounts of work, in order to tire the heart. We employ in these people the 25-pound steel bar which is lifted from the floor to the shoulder, and above the head until the arms are fully extended. Then it is lowered quickly to the floor and raised again above the head. An adult will raise the bar between 6–7 feet, performing thus between 150 and 175 foot-pounds with each raising. In addition to the work arising from the bar movement, the raising of the trunk of the body each time from a stooping to an erect position is equivalent to a certain number of foot-pounds. The exact number is very difficult to estimate, but from some comparative experiments we are now carrying out it apparently lies between 40 and 50 per cent. of the body weight. That is, a man who weighs 150 pounds does between 60 and 75 foot-pounds of work each time that he raises his body from a stooping to an erect position.

The testing of these hearts is necessarily at the present time a comparative matter, and we are unable to obtain absolute values, unless we have a bicycle ergometer at our disposal.

We are much indebted to Dr. Horatio B. Williams, of the department of physiology of Columbia University, for his assistance in supervising the experiments conducted with the bicycle ergometer and for many valuable suggestions made during the course of the work outlined above.

30 (1094)

## The influence of infantile scurvy on growth (length and weight).

By ALFRED F. HESS, M.D.

[*From the Board of Health Laboratories, New York City.*]

Infants fed on milk that has been pasteurized (heated to 145° F. for 30 minutes) develop scurvy, provided fruit juices or other anti-scorbutic food is not added to their diet. This scurvy is of a

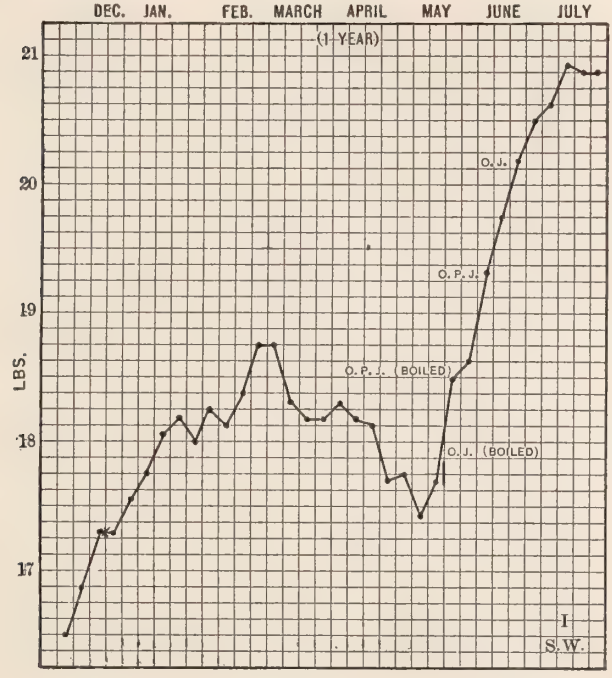


Chart 1.

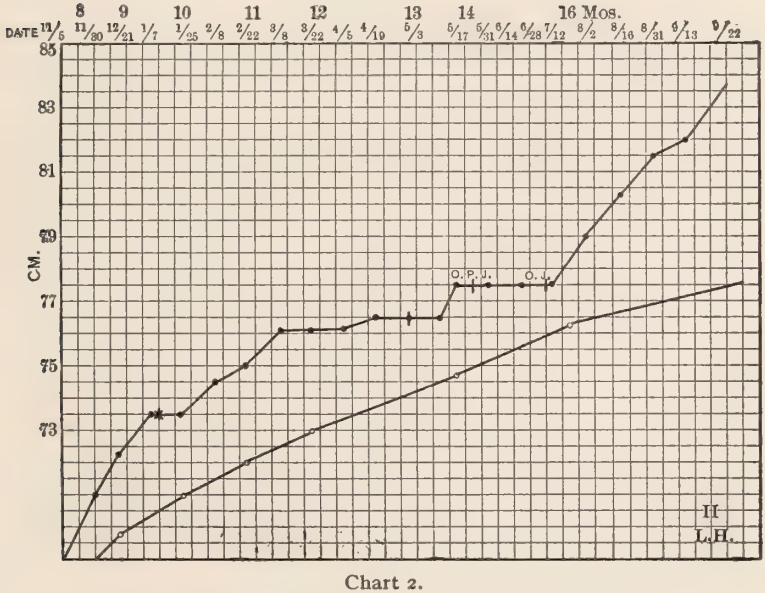


Chart 2.

O. J. = orange juice. O. P. J. = orange peel juice.

* Orange juice stopped.

subacute type, requiring two or more months to manifest itself. Under otherwise excellent surroundings it developed among infants in an asylum, where the babies are weighed daily and measured fortnightly. Its effect on growth could thus be carefully followed. In this connection, three periods may be distinguished: one of about three months when orange juice was given, a second of about four months when the infants did not obtain fruit juice, and a third extending over about a half year, where they once more obtained orange juice.

It was found that in almost every instance a gradual failure to gain in weight accompanied the absence of orange juice from the diet, and that this failure was corrected when the juice of the orange, or the orange peel (even though boiled) was again given (Chart 1). In most cases increase in length was likewise retarded by the scorbutic condition and this stunting was corrected by means of the fruit juices; a notable instance may be seen in Chart 2.

31 (1095)

## The action of the depressor nerve on the pupil.

By JOHN AUER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

Stimulation of the depressor nerve in white rabbits, narcotized by the subcutaneous injection of 5–10 milligrams of morphine sulphate per kilo, usually causes a definite diminution of the pupil. This contraction in typical cases is composed of two stages: a sharp, prompt, short, initial contraction followed by a slower gradual one. Often only the initial contraction is observed, at other times only the slower gradual contraction.

The initial contraction, when present, is obtained as soon as the nerve is stimulated, before the blood pressure begins to fall. The slower contraction occurs while the bloodpressure is falling, and the iris blanches at the same time.

Stronger stimuli are necessary to cause this contraction of the

pupil than suffice to bring on the characteristic drop of blood-pressure. A strong fall of bloodpressure due to a moderate depressor stimu'ation does not cause any alteration of the pupil.

Stimulation of one depressor may cause a contraction of the pupil on the opposite side.

This pupillary effect cannot be obtained with the same certainty as the fall in bloodpressure. After several successful trials, the pupil usually fails to respond for a while.

The two depressors vary in their pupillary effect; one may yield excellent pupillary contractions, the other one none at all.

The stimuli used were rarely longer than three to five seconds; the strength 100–150 mm. coil distance (Petzold coil).

Section of the sympathetic nerve, or extirpation of the superior cervical ganglion, the depressor of the same side being stimulated several days later, exerts no appreciable effect on the result. The reflex therefore seems to act on the third nerve chiefly, if not entirely.

In addition to this pupillary effect, depressor stimulation at times causes a short wink or a more or less prolonged retraction of the bulbus.

It must be added that a strong winking (closure of the lids being prevented by a speculum) usually causes a very short sharp contraction of the pupil, the Piltz-Westphal phenomenon. This contraction is, however, much more rapid than what has been described as the initial contraction on depressor stimulation; moreover, the initial contraction is frequently obtained without any sign of winking.

In rabbits anesthetized by ether or which have been allowed to recover from the ether, the depressor pupil effect was not obtained. An increase of reflex irritability is apparently necessary in order to obtain the depressor pupillary contraction.

32 (1096)

# Studies on so-called protective ferments—X. Some suggestions as to the etiology and treatment of eclampsia.

By J. BRONFENBRENNER.

[*From the Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.*]

PRELIMINARY COMMUNICATION

Many theories concerning the etiology of eclampsia have been advanced. For certain of them there is more or less experimental and clinical evidence. The treatment, however, remains purely empirical. Each theory has as its basis that the eclamptic seizure is due to toxin developed as the result of pregnancy. As to the origin of this toxin, the authors vary to as great a degree as do the theories. The development of this toxin may be due to:

(*a*) A growing ovum and its metabolic products.

(*b*) Functional changes in the liver resulting from pregnancy.

(*c*) General metabolism being affected during pregnancy with the result that the food stuffs are not cared for properly.

(*d*) The advent of the study of parenteral digestion of foreign protein by specific ferments suggested another possibility that the toxins originate from the detached elements of the placenta as a result of their specific parenteral digestion.

The numerous attempts that were made to demonstrate by experiment the presence of this toxin in the blood were not successful. My recent experiments[1, 2] have demonstrated that pregnant serum can be rendered toxic to homologous animals by being kept for a definite time in contact with placental tissue. The toxic symptoms produced by the introduction of such a serum into an experimental animal closely resemble those generally known as anaphylactic shock. On the other hand, the injection of soluble placental protein into the blood circulation of pregnant guinea-pigs produces similar symptoms.[3] Further experiments

[1] Bronfenbrenner, *Bioch. Bull.*, 1914, IV, No. 13, p. 87.

[2] Bronfenbrenner, PROC. SOC. EXP. BIOL. AND MED., 1914, Vol. XII, p. 48.

[3] Bronfenbrenner, *Journ. Exp. Med.*, 1915, XXI, p. 480.

have shown that the mechanism of the formation of toxin is that of autodigestion of the serum with the formation of toxic split products.[4] This autodigestion is made possible through the liberation of non-specific proteolytic enzyme normally present in the blood of every animal.[5] These results seem to suggest another theory of the causation of eclampsia.

The development of the ovum is accompanied by metabolic changes in the body directly connected with the requirements of this new growth. In addition, the penetration into the general circulation of detached cells of the developing embryo together with the metabolic products of the growing fetus, sets up a new specific process of parenteral digestion of these substances in the body of the mother. As the gestation progresses and the products of the new growth repeatedly penetrate the general circulation they cause the appearance of specific antibodies of a cytolytic nature. My experiments suggest that such antibodies by combining with the antigen change the balance of the blood constituents so as to allow the liberation of the serum trypsin, which in turn digests the antigen circulating in the blood.[6] This trypsin, however, is capable of digesting also the serum itself. The products of such autodigestion of serum are very toxic and when they occur in early pregnancy they may induce symptoms of nausea, dizziness, general depression and so forth. If the amount of toxin produced by this autodigestion of serum goes beyond the limit of tolerance for the individual, acute symptoms of anaphylaxis result and we witness the eclamptic convulsions.

Normally the intoxication from the split products of such autodigestion is prevented by at least two independent processes. One is the overproduction of antitrypsin, which prevents the excessive autodigestion of serum by neutralizing the proteolytic ferments; the other is the elimination of toxic substances through the liver and kidneys which retain these toxins and, therefore, show signs of local involvement long before the general symptoms are noticed. That the antitrypsin of the blood is involved in this process was shown in this laboratory by the actual measurements[7]

[4] Bronfenbrenner, PROC. SOC. EXP. BIOL. AND MED., 1914, XII, p. 7.

[5] Bronfenbrenner, *Journ. Exp. Med.*, 1915, XXI, p. 221.

[6] Bronfenbrenner and Scott, PROC. SOC. EXP. BIOL. AND MED., 1915, XII, p. 137.

[7] Full records of these experiments will be published in the near future.

of the antitryptic index in a series of cases of eclampsia. These measurements revealed the fact that in all cases of eclampsia the patients show a very low antritryptic index. In cases where the surgical intervention took place, the antitryptic index shows very marked rise after the emptying of the uterus.

In cases where the convulsions were intermittent, spaced with periods of comparative rest, a very noticeable curve of antitrypsin in the blood could be established. The lowest points on this curve corresponded very suggestively with the convulsions. It is the high amount of antitripysin in the blood, which is so characteristic in normal pregnancy, that prevents usually the excessive autodigestion and resulting eclampsia. In abnormal cases, where the antitrypsin is unusually low, or, where the elimination is insufficient, or where both conditions are present at the same time, the general intoxication occurs.

A critical review of the methods of empirical treatment seems to me to offer considerable additional support to the above suggestion of the etiology of eclampsia. The methods of treatment of eclampsia may be considered under four headings: (1) Administration of anesthetics and sedatives, such as ether, chloroform, morphin, chloral, and similar drugs. (2) Serum treatment. (3) Eliminative treatment. (4) Surgical treatment.

Experiments carried out in this laboratory[8, 9] have shown that the administration of anesthetics and sedatives is followed by a more or less pronounced rise of antitrypsin in experimental animals, thus suggesting the possibility that the therapeutic value of these substances might be dependent on their ability to cause the rise of antitrypsin.

As for the serum treatment, Wolff[10] suggested that the therapeutic value of the injection of the serum from normal pregnant individuals, has a decided therapeutic effect, which is due to the specific protective ferments contained in the injected serum. The fact, however, that on the one hand normal human serum from non-pregnant individuals, as well as normal horse serum, were both successfully used for this purpose;[11] on the other hand, our

[8] Bronfenbrenner, PROC. SOC. EXP. BIOL. AND MED., 1915, XII, p. 110.

[9] Bronfenbrenner and Schlesinger, *Journ. Exp. Med.* (in press).

[10] Wolff, *Berl. klin. Woch.*, 1913, No. 13, p. 1661.

[11] Freund, *Deut. med. Woch.*, 1913, No. 24, p. 1179.

recent findings, showing that there are no specific ferments in pregnancy,[5, 12] exclude the explanation given by Wolff. It is known, however, that every normal serum is antitryptic, and especially during pregnancy the antitryptic titer of the serum is very high. Therefore, it is possible that the therapeutic value of such serum depends on its antitryptic property.

Eliminative treatment and surgical intervention remove respectively the toxic split products of autodigestion, and the source of the invading material, which alone sufficiently explains their action.

Considered from the above point of view, the relation between the etiological factors involved and the clinical treatment of eclampsia may be thus graphically represented:

TABLE.

Gestation—Antibody Formation.

| Antibody Formation Normal | Antibody Formation Deficient. Formation of various amounts of toxic split products. |
|---|---|
| Normal Pregnancy. | Early Symptoms of Toxemia. |

| Good Elimination. | | Poor Elimination. | |
|---|---|---|---|
| Sufficient Antitryp. | Deficient Antitryp. | Sufficient Antitryp. | Deficient Antitryp. |
| Slow autodigestion. Poisons eliminated. | Autodigestion pronounced. Poisons eliminated. | Slow autodigestion. | Marked autodigestion. |
| Symptoms very mild. | Symptoms more marked. Possible involvement of liver and kidneys. | Mild symptoms. Possible involvement of liver and kidneys. | Severe symptoms. Acute involvement of liver and kidneys. |
| No treatment. | Antitryptic serum treatment. | Eliminative treatment. | Combined antitryptic and eliminative treatment. Possibly surgical intervention. |

[12] Bronfenbrenner, PROC. SOC. EXP. BIOL. AND MED., 1914, XII, p. 3.

33 (1097)

## The availability of certain indicators in the determination of gastric acidity.

By CHESTER C. FOWLER, OLAF BERGEIM, and P. B. HAWK.

[*From the Department of Physiological Chemistry, Jefferson Medical College.*]

A comparative study was made of certain indicators which have been used in the titration of gastric juice for free and total acidity and for the colorimetric determination of the H ion concentration of the same. The findings apply particularly to titrations where small amounts of gastric juice are available and many determinations must be carried out.

Phenolphthalein was the most satisfactory indicator for total acidity in spite of the fact that values obtained with it are slightly high, because the end point is definite and speed and accuracy are attainable with its use while the use of litmus or alizarin requires more time and the end points cannot be determined with accuracy.

For the titration of free acidity the iodometric method was found most useful. In the higher acidities dimethylaminoazobenzene may be used with an equal degree of accuracy and may be preferred where much starch is present. Congo red possessed no advantages over the foregoing in the determination of free acid but gives high results and the end point is not sufficiently sharp.

Colorimetric H ion concentration determinations when applied to gastric contents containing varying quantities of protein and salts have so far proven inaccurate and unavailable for following changes in reaction during digestion. Better results have been obtained after partial removal of protein by short dialysis through collodion sacs according to a procedure similar to that used by Levy, Rowntree, and Marriott[1] for blood. The use of adsorbents for protein and substitutive colloid in standards did not give completely satisfactory comparative results, though attempts are still being made in this direction.

[1] Levy, Rowntree, and Marriott, *Arch. Int. Med.*, 16, 389, 1915.

34 (1098)

## An interrelationship between calcium and antithrombin in blood coagulation.

By ALFRED F. HESS, M.D.

[*From the Board of Health Laboratories, New York City.*]

Experiments with dilutions of hirudin show that the stronger the antithrombic solution, the less calcium is needed for optimum coagulation; in other words that, to a certain extent, they bear an inverse ratio to each other. In the accompanying table we see tests of plasma containing hirudin (1 : 40,000) to which 3, 4, 5 and 6 drops of a calcium solution were added. We may note that where only 2 drops of hirudin were added, the clotting-time was in all four instances the same; when three drops were added the time was delayed where 6 drops of calcium were used; and where four drops of hirudin were present, coagulation was markedly delayed in both the tubes containing 5 and 6 drops of the calcium solution.

| | Hirudin Solution (Drops). | | | Min. |
|---|---|---|---|---|
| | 2 | 3 | 4 | |
| $CaCl_2$ 3 drops. | − | − | − | 4 |
| | ++ | + | + | 8 |
| | +++ | +++ | + | 12 |
| | | | + | 16 |
| | | | ++ | 20 |
| | | | +++ | 24 |
| $CaCl_2$ 4 drops. | − | − | − | 4 |
| | ++ | + | + | 8 |
| | +++ | +++ | + | 12 |
| | | | ++ | 20 |
| | | | +++ | 24 |
| $CaCl_2$ 5 drops. | − | − | − | 4 |
| | ++ | + | − | 8 |
| | +++ | +++ | + | 12 |
| | | | ++ | 28 |
| | | | +++ | 44 |
| $CaCl_2$ 6 drops. | − | − | − | 4 |
| | + | + | + | 8 |
| | ++ | ++ | + | 12 |
| | +++ | +++ | + | 20 |
| | | | ++ | 34 |
| | | | +++ | 66 |

35 (1099)

## Observations on cholesterol-fed guinea pigs.

By **C. H. BAILEY, M.D.** (by invitation).

[*From the Pathological Laboratory of Stanford University Medical School.*]

The following experiments were done to test the possibility of the production of atheroma of the aorta in guinea pigs by cholesterol feeding, and also to test certain theories which have been advanced as to the importance of factors, other than the cholesterol, in the production of this type of atheroma.

Four guinea pigs were fed on daily doses of 0.1 to 0.5 gm. of cholesterol dissolved in cotton seed oil for periods of 18 to 72 days. These animals, like rabbits similarly fed, show an enlargement of the adrenals, and an abundant deposit of anisotropic fat in the liver and spleen, the situation of this fat being similar to that previously described in these organs in the rabbit. An occasional guinea pig in this and the following experiments showed focal areas of degeneration in the cortex of the adrenal with a deposit of calcium. The aortas show no gross lesions. Microscopically there are found small patches of fatty infiltration in the intima and upper media. The characteristic proliferation and subsequent degeneration seen in the rabbit were entirely lacking. The feeding periods were too short to conclude that such tissue reaction might not ultimately result. One guinea pig which received 20 g. of cholesterol in 72 days (15.1 g. in the last 40 days) would seem however quite comparable with a rabbit, previously reported, which showed pronounced atheroma after receiving 13.7 g. in 37 days. From these experiments and others which follow it can at least be concluded that a longer period and larger doses are necessary for the production of an atheroma in the guinea pig than in the rabbit.

A guinea pig receiving 13.4 g. cholesterol without oil in 51 days showed some adrenal enlargement, but no anisotropic fat could be found in liver, spleen, or elsewhere. Knack, because of failure to produce atheroma in a rabbit with cholesterol alone, concludes that previous injury to the aorta is necessary before a deposit of

anisotropic fat occurs, and that such injury is produced by oil. If such injurious effect is exercised by cotton seed oil, it is also exercised by various oils which have been used as vehicles in the administration of cholesterol, and also by some substance in egg yolk other than cholesterol. A rabbit may moreover be fed large daily doses of cotton seed oil over a long period without the production of any demonstrable lesion of the vessels. It would therefore seem more probable that the importance of the fat lies in the fact that it supplies to the diet of these animals, normally low in fat, an essential factor for the formation of esters, and thus enables the absorption of cholesterol in large amounts, and possibly also that it facilitates the formation of some compound with cholesterol, in the process of metabolism, which is toxic to the vessels.

Anitschkow and Aschoff believe that the production of atheroma in cholesterol-fed rabbits is aided by raising the blood pressure by mechanical or chemical means. Since the production of cholesterol atheroma in rabbits is so rapid without resort to artificial means of changing the blood pressure, the guinea pig seemed a more suitable animal for testing this theory. A guinea pig received daily doses of cholesterol in oil, 12 gm. in all, for a period of 82 days. During the last 32 days of the feeding he was suspended head down for 15 to 40 minutes daily. Another received 4.7 g. in 44 days and was suspended 20 to 40 minutes daily for the last 38 days. The distribution of the fat was the same as in the preceding experiments, the amount deposited in the aorta was not greater, and tissue reaction was similarly lacking. The same negative results were obtained in 2 guinea pigs receiving daily subcutaneous injections of pituitrin during the feeding.

Four guinea pigs were put on a feeding of cholesterol in oil 26 days after the last of 2 injections of uranium and continued on this feeding for 43, 80, 83, and 88 days. Since it is believed that the cholesterol kidney lesions previously described by the author in rabbits are dependent on a preëxisting spontaneous nephritis, it was hoped in this way to obtain similar lesions from the deposit of cholesterol in the interstitial lesions of chronic uranium nephritis. The kidneys of all these guinea pigs showed a small amount of anisotropic fat, while in only one of the above reported 9 guinea pigs could any of this fat be found in the kidneys. The fat was

present in a few large cells, probably of endothelial type, in the scars, but the lesions seen in rabbits were not obtained. There was also some fat in the glomerular tufts. The latter observation might be considered as evidence that uranium produces a vascular lesion and that such a preëxisting lesion facilitates the absorption of anisotropic fat as argued by Knack. The deposit of fat in the aortas of these animals however was not increased.

36 (1100)

**Lesions produced in rabbits by repeated intravenous injections of living colon bacilli.**

By **C. H. BAILEY, M.D.** (by invitation).

[*From the Pathological Laboratory of Stanford University Medical School.*]

Of a series of rabbits injected intravenously with a strain of colon bacillus every 3 or 4 days over considerable periods, the 7 animals which withstood this treatment longest, namely 88, 98, 102, 113, 115, 116, and 142 days, showed pronounced lesions in the kidneys, spleen, and liver. In the kidneys there is produced a hyaline and fibrous thickening of the vascular loops of the glomeruli with the formation of hyaline bodies in the tufts and occasional adhesions between the tufts and glomerular capsules. The tubular epithelium shows more or less degeneration and many casts are present in the tubules. The interstitial connective tissue shows a beginning cellular thickening, apparently not due to the spontaneous nephritis frequently seen in rabbits.

The livers show in certain cases central necroses with hyaline degeneration of the liver cells about these areas and elsewhere. In two cases there is deposited between the rows of liver cells in the middle and peripheral portions of the lobules a homogeneous amyloid-like substance. The livers in all cases show a more or less marked cellular increase of the periportal connective tissue—the latter possibly a spontaneous lesion.

The spleens show a fibrous thickening of the reticulum of the pulp with some hyaline formation. The most striking lesion is a formation of connective tissue with much amyloid-like material about the peripheries of the Malphigian bodies, in cases almost replacing these structures.

The appearance of the homogeneous substance described and its distribution naturally suggest amyloid. Attempts to produce the typical staining reactions of this substance failed however, possibly owing to the fact that the material had unfortunately been fixed in Orth's fluid before the attempt was made.

37 (1101)

## The determination of amino nitrogen in urines containing glucose and albumin.

By DONALD D. VAN SLYKE.

[*From the Hospital of the Rockefeller Institute for Medical Research.*]

*Albumin.*—For removal of the albumin, we have found Welker's aluminum hydrate method very satisfactory. For urines containing large amounts of albumin, however, it is advisable to first coagulate with heat and acetic acid; then add an equal volume of the 0.5 per cent. aluminum hydrate suspension. The amino nitrogen is determined in an aliquot part of the filtrate.

*Glucose.*—Glucose interferes in two ways with the previously described method for determining amino nitrogen in the urine. If the urea is removed by treating the urine with urease, glucose combines with some of the ammonia and forms small amounts of an amine, which makes the results for amino nitrogen come out too high.

On the other hand, if a solution of an amino acid is concentrated on the water bath with glucose, a condensation occurs, with disappearance of amino nitrogen.

Both difficulties are obviated as follows: The urine is kept acid during action of the enzyme by the addition of three or four volumes of charged $CO_2$ water. After the action of the enzyme is completed at room temperature, the glucose is removed by adding sufficient copper sulphate solution to form an insoluble compound containing five molecules of copper hydrate to one of glucose. This compound is precipitated by the addition of a slight excess of calcium hydrate. The alkaline filtrate is concentrated in a vacuum, removing the ammonia, and the free amino nitrogen determined. In case albumin is present in a diabetic urine, this treatment removes the albumin along with the glucose.

38 (1102)

## On continuous insufflation in fowls. A demonstration.

By **A. L. MEYER** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

For many years this laboratory was interested in continuous insufflation which was carried out on mammals. At this meeting we wish to demonstrate apnœa produced by continuous insufflation in fowls. As you know, in these animals the bones are connected with the air sacs and the lungs. In this chicken, air is driven through both humeri and it escapes through a tracheal cannula. When the air is driven under sufficient pressure the respiratory movements are entirely abolished; the thorax stands still mostly in an exaggerated state of inspiration. This standstill may be sustained for two hours and longer. By this method the organism is liable to be washed out of its $CO_2$ content more thoroughly than by any other method of artificial respiration or forced respiration. Nevertheless, the animals appear to be in a good condition with no symptom which could be interpreted as "shock". Furthermore, when the continuous insufflation is interrupted, one of the following conditions may follow, according to the duration of the standstill, the degree of pressure, and to the gas used. Either the inspiratory state may be converted at once into a continuous expiratory standstill (apnœa vera), lasting 20 to 50 seconds and gradually attaining the amplitude of the original respiratory oscillations; or the state of the inspiratory standstill continues, in a somewhat diminished degree, for many seconds before it is converted, abruptly or gradually, into an expiratory standstill. We shall mention briefly the facts that an admixture of ether to the insufflated air invariably prolongs the expiratory after-effect, and that an admixture of $CO_2$ (3 per cent.) prevents the standstill even during insufflation.

*In fowls the expiration is normally of an active type* and the expiratory standstill can only mean that after the interruption of the insufflation the expiratory muscles get temporarily into a state

of strong tonic contraction. Since the continuous insufflation washes out a good part of the normal content of $CO_2$ and since the effect and the after-effect of the insufflation practically consist at all times in a tonic contraction of either the inspiratory or the expiratory muscles, the conclusion seems warranted that a *reduction of $CO_2$ in the blood does not act as a reduction of a stimulus below the threshold value but, on the contrary, it serves as a stimulus for the production of a tonic contraction of the respiratory muscles, while the addition of $CO_2$ assists in the maintenance of the respiratory rhythm.*

39 (1103)

## On the production of hyperglycæmia and glycosuria by magnesium salts.

By I. S. Kleiner and S. J. Meltzer.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute.*]

In their experiments on the action of magnesium salts, Meltzer and Auer observed that after subcutaneous injections of magnesium sulphate the urine of rabbits contains a reducing substance. Underhill and Closson, who later noticed the presence of hyperglycæmia after an intravenous injection of magnesium sulphate, ascribed the hyperglycæmia to the asphyxia which the magnesium salts produced in their experiment.

In a series of experiments which we have recently carried out on dogs, all the animals had from the beginning to the end of the experiment either intratracheal insufflation or the usual artificial respiration. The occurrence of asphyxia was thus excluded. The operative part was done under local anesthesia. In most of the experiments an $M/4$ solution of magnesium sulphate was injected intravenously. There was a considerable increase of the sugar content of the blood after the infusion practically in all experiments. In most cases the original glycæmia did not exceed 0.13 per cent., while at the end of the injection or some time later, the sugar content of the blood was often as high as 0.4 per cent. and even higher. In blood taken about an hour and a half after the end of

an injection the glycæmia was found often to have dropped to the original content.

There can be no doubt that magnesium sulphate produces considerable hyperglycæmia which is not due to asphyxia; it is produced in some way specifically by the magnesium salt. Sodium sulphate does not affect the normal glycæmia.

It is a noteworthy fact that the glycosuria was very little marked and far under proportion to the hyperglycæmia. Glycosuria was often entirely absent and when present it never reached even ½ per cent. The intravenous injection of magnesium sulphate also produced very little diuresis.

# SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

**Seventy-second meeting.**

*University and Bellevue Hospital Medical College, January 19, 1916.*

*President Lusk in the chair.*

40 (1104)

## On the relation of blood sugar to glycosuria in diabetes mellitus.

By **A. A. EPSTEIN** (by invitation).

[*From the Department of Pathology of Mt. Sinai Hospital, New York.*]

An extensive study on diabetic individuals and on animals in which diabetes was produced by the removal of the pancreas, reveals the fact that the current belief concerning the non-existence of a relationship between hyperglycemia and glycosuria is erroneous.

The error in the conclusions heretofore reached concerning this matter arises from several circumstances, chief of which are: First, the failure to recognize the fact that the blood volume in animals and man is capable of undergoing considerable variations, which affect the concentration and the total amount of the sugar present in the blood; second, the employment of the "percentage" of sugar found in the blood as a measure of hyperglycemia; third, comparing the "percentage" of sugar in the blood as found by single or isolated determinations, with the quantity of sugar eliminated in the urine in a given period of time.

Evidence is adduced to show that the volume of blood circulating in the body is capable of variation spontaneously, and as a result of the addition or abstraction of fluid. The degree of variation of the blood volume (*i. e.*, the relative blood volume)

may be determined by means of the hematocrit without resorting to the use of a method which measures the total blood volume. This is accomplished by establishing the changes that occur in the proportion of cells in the blood from time to time and computing therefrom, the alteration in the blood volume.

Variations in the blood volume appear to be capable of affecting the percentage relations of the different constituents of the blood, including sugar. This circumstance necessitates a consideration of the blood volume in the study of the sugar content of the blood; for a change in the blood volume may alter the percentage concentration of the sugar and thus mask or modify an actual increase or decrease in the amount of sugar present. Evidence is furnished showing that the percentage of sugar in the blood may rise or fall, as a result of change in the blood volume (bleeding, anesthesia, sweating, ingestion of fluid), but the total amount may remain unchanged. It is also shown that an increase in the total amount of sugar may occur synchronously with an increase in the blood volume (through dilution), causing little or no rise in the percentage of sugar.

In view of these facts it appears necessary to define hyperglycemia as an increase in the total amount of blood sugar over the normal, and not merely an increase in concentration or percentage. Thus it is possible to have a hyperglycemia even when the percentage of sugar is normal or below normal.

The blood sugar is subject to very rapid changes. A single examination of the blood sugar can not be used as a measure of the sugar content for any period of time, no matter how brief. Frequent estimations are therefore necessary, and the mean of two or three estimations must be used to represent the probable quantity of sugar in the blood for a given period of time. By following this plan in the examination of the blood of diabetic and non-diabetic individuals, it is found that the occurrence of glycosuria is intimately connected with a hyperglycemia, *i. e.*, an increase in the total amount of sugar in the blood.

In considering the quantitative relations between the glycosuria and the blood sugar in diabetes mellitus, the "hyperglycemia" is regarded as the sum of the greatest amount of sugar that can be present in the blood without giving rise to glycosuria,

plus an excess.[1] By comparing the urinary output of sugar in a given period of time with the "excess" of sugar present in the blood, a definite mathematical relation is found to exist between the percentage of sugar in the urine, and that in the blood. The proportion between the two tends to approach a constant, in one and the same individual, on a given day. This applies to individuals with normally functionating kidneys. In those with defective kidneys there is no parallelism. The hyperglycemia in such individuals is usually greater in proportion to the glycosuria than it is in those with normally functionating kidneys.

Diuresis in diabetes mellitus plays an important rôle in determining the total amount of sugar eliminated in the urine, but has no influence on its concentration or percentage.

41 (1105)

## The relation of the sugar content and concentration of the blood to urine formation. (Preliminary report.)

By **E. M. EWING.**

[*From the Laboratory of Physiology, University and Bellevue Hospital Medical College.*]

The present experiments were performed in an effort to establish a standard of comparison for further experiments involving the study of the fate of sugar injected into the circulatory system under various conditions.

Various amounts of a dextrose solution (40 gm. in 40 c.c. water) were injected into the femoral veins of dogs under local anesthesia, and 20 c.c. of blood drawn from the femoral artery at 15-minute intervals. Twenty c.c. of citrated dog's blood previously prepared were injected into the artery immediately afterward, thus maintaining the normal concentration of the blood as indicated by the specific gravity and hemoglobin percentage. The specific gravity, hemoglobin and sugar content of the blood were determined, and also the urine obtained by catheter was

[1] The largest amount of sugar which may be present in the blood (with constant blood volume conditions) without giving rise to a glycosuria, is estimated at 0.1 per cent. In determining the excess of sugar in the blood, 0.1 per cent. is deducted from the values of blood sugar obtained after correction for blood volume.

measured and its sugar content estimated. Specific gravity was determined with a 10 c.c. pycnometer; hemoglobin with the Sahli instrument; blood sugar and urine sugar by the Lewis-Benedict and Benedict methods, respectively.

I. Fisher and Wishart have presented evidence that (contrary to Starling's hypothesis) after the ingestion of 50 gm. of dextrose by a dog, there is a diminished formation of urine in spite of a dilution of the blood, as indicated by hemoglobin determinations.

Similar results were obtained also in the present series of experiments after *intravenous* injections, estimations of the specific gravity as well as the hemoglobin content of the blood being made. The following experiments indicate clearly that simple increase or decrease in the concentration of the blood following injection of crystalloids does not necessarily result in a corresponding increase or decrease in urine formation, and that there may be a definite diuresis without any material change in the concentration of the blood.

| Exper. | Injected. | Change in Sp. Gr. in 30 Min. | Urine in 30 Min. | Rate per Hour. |
|---|---|---|---|---|
| May 14, 7 K.... | 40 gm. dextrose, 10 min. | 1,0570[1] to 1,0592 | 14 c.c. | 28 c.c. |
| May 24, 9 K.... | 30 c.c. water, 30 min. | 1,0555 to 1,0551 | 9 c.c. | 18 c.c. |
| May 27, 8 K.... | 13 gm. dextrose, 30 min. | 1,0660 to 1,0631 | 3.25 c.c. | 6.5 c.c. |

II. Complete results of the experiments would be too bulky for publication, but the following summary will show the relation of blood sugar, blood concentration and diuresis. The weights of the animals are indicated along with the dates of the experiments; the figures for the blood sugar represent the highest points reached during the chosen periods; the maximum changes in the specific gravity of the blood during the period are indicated, and the urine formation is expressed in c.c. per hour.

| Exper. | Injection. | Blood Sugar. | Sp. Gr. Decreased. | Urine Rate per Hr. |
|---|---|---|---|---|
| May 14, 13 K..... | 40 gm. in 1 hr. | .57 | 1,0620 to 1,0580 | 33 c.c. |
| May 24, 9 K..... | 20 gm. in ½ hr. | .55 | 1,0630 to 1,0610 | 50 c.c. |
| May 21, 10 K..... | 20 gm. in 1 hr. | .28 | 1,0627 to 1,0607 | 10.5 c.c. |
| June 4, 9 K..... | 13 gm. in ½ hr. | .50 | 1,0573 to 1,0550 | 12 c.c. |
| June 5, 9 K..... | 13 gm. in 5 min. | .68 | 1,0570 to 1,0546 | 55 c.c. |
| May 27, 8 K..... | 13 gm. in ½ hr. | .57 | 1,0660 to 1,0632 | 7 c.c. |
| May 28, 8 K..... | 13 gm. in 5 min. | .6 | 1,0657 to 1,0610 | 64 c.c. |

[1] This change occurred 30 minutes after the injection, being preceded by a marked fall (as in Starling's experiment).

A glance at the above table will suffice to show that the flow of urine bears but slight relation to the degree of dilution of the blood as indicated by the specific gravity determinations. Diuresis is apparently the greatest when the percentage of blood sugar is highest. On the other hand, there is no question but what diuresis is absent in experiments showing excessively high blood sugar.

For the experiments of May 27 and 28, and of June 4 and 5, the same animals were used, the experiments differing only in that the rate of injection was rapid in one case and slow in the other. Here it seems that the presence of diuresis depends upon the rapidity of injection, and not upon the blood sugar content, which in both cases is excessively high.

III. The quantity of sugar per c.c. of urine was surprisingly *constant* when the blood sugar was above 0.4 per cent., *i. e.*, during the period following injection. In a series of experiments with the blood sugar varying from 0.4 to 0.6 per cent. the sugar per c.c. of urine varied from .045 to .052 gm., independently of the presence or absence of diuresis. Toward the end of the experiment, however, with blood sugar falling, and diuresis coming to an end, the sugar per c.c. of urine rises steadily. For example, in one experiment, at different periods, the blood sugar percentage was .5, .35, and .28; while for corresponding periods the urine sugar per c.c. was .05, .07, and .08 gm.

42 (1106)

## A method for the estimation of levulose in presence of glucose.

By Leon Loewe (by invitation).

[*From the Department of Chemistry, Cornell University Medical College, New York City.*]

The reagent consists of 0.2 per cent. aqueous solution of orcein (Kahlbaum) and 85 per cent. phosphoric acid (Eimer and Amend) in separate containers.

The qualitative test is carried out as follows: to 1 c.c. of the solution under investigation in a test tube, add from 6 to 8 drops of the orcein solution and 1 c.c. of the phosphoric acid. The test tube

is heated to boiling over a free flame and then placed in a boiling water-bath for ten minutes. If levulose is present, a yellow color appears which is deeper for greater concentrations of the sugar. A yellow precipitate settles out on cooling. Upon the addition of alkali (KOH or NaOH) sufficient to neutralize the phosphoric acid, the yellow color changes to a distinct orange. The characteristic orange color developed was made the basis for the quantitative determination of levulose.

The procedure for the quantitative test is essentially the same. The standard for colorimeter comparison is a definite known solution of levulose in distilled water. The strength of the standard is arbitrary but should be as near the concentration of the unknown solution as possible to favor a more accurate comparison. Hence the advisability of performing a preliminary test to determine the optimum standard. The standard and unknown solutions are similarly treated, to 1 c.c. of each in separate test tubes add 8 drops of the orcein solution and 1 c.c. of phosphoric acid. The solutions are, as before, boiled over a free flame and heated in a boiling water-bath for ten minutes. The test tubes are then removed and the contents of each are transferred quantitatively to separate ten c.c. volumetric flasks and made up to the mark with 5 *N* NaOH. The solutions are at once placed in the colorimeter chambers and the orange colors compared.

## RESULTS.

*Qualitative.*—Levulose was detected in 1 c.c. of a 0.005 per cent. solution.

A positive reaction was obtained from 0.5 c.c. of a 0.01 per cent. solution of levulose in the presence of 0.5 c.c. of a 20 per cent. solution of dextrose.

Maltose, lactose, galactose, and R. arabinose in solutions of various strengths did not interfere with the test.

Cane sugar yielded a positive test, due to the presence of levulose from the hydrolytic cleavage of the sugar in the presence of the phosphoric acid.

*Quantitative.*—The orange color was found to be characteristic for the different concentrations of levulose. Uniformly good results were obtained with unknown solutions in the presence of various amounts of glucose.

Levulose was added to urine and subsequently quantitatively recovered after treatment of the urine with lead acetate to remove coloring matter and inorganic salts.

43 (1107)

**A method for obtaining suspensions of living cells from the fixed tissues, and for the plating out of individual cells.**

By PEYTON ROUS and F. S. JONES.

[*From the laboratories of The Rockefeller Institute for Medical Research.*]

The method depends on the ability of living tissue cells to withstand tryptic digestion. It is applicable to such cells as will proliferate *in vitro*. Bits of tissue are cultivated in plasma, according to Burrows's modification of Harrison's technic, and when growth is well under way the preparation is flooded with trypsin dissolved in Locke's solution. Under the influence of this fluid the growing cells contract into spheres, and with the digestion of the fibrin network they are liberated. Suspensions of individual cells are thus obtained comparable to suspensions of leukocytes. When washed and plated anew in plasma the cells put forth processes and proliferate. The digestion and plating can be repeated. The method is most successful with tissues that grow loosely in strands or a network,—sarcoma, choroid, endothelium (?), connective tissue,—in distinction from those proliferating in sheets, as do the epithelial tissues. The cells of these latter are usually liberated in clumps, not as individuals.

44 (1108)

**The carbon dioxide content of blood and alveolar air in obstructed expiration.**

By E. D. FRIEDMAN and H. C. JACKSON.

[*From the Laboratory of Physiology of University and Bellevue Hospital Medical College.*]

In the course of a study of the effects of respiration on the circulation and more especially the modification of these effects in

asthma and emphysema, we noted (1) low pulse pressure in those cases uncomplicated by atherosclerosis, probably due to a diminished systolic volume; (2) the loud pulmonic second sound pointing towards increased resistance in the lesser circulation; (3) polycythemia, possibly a teleologic phenomenon designed to compensate for a lessened minute volume output from the left ventricle; (4) enlarged veins in the neck and cyanosis suggesting a certain amount of venous stasis. These observations led us to believe that there was considerable circulatory disturbance in conditions associated with obstructed expiration.

Due to the lack of adequate clinical material, we decided to study this subject in the experimental animal.

Dogs were used. A saturated solution of chloretone and morphine was employed for inducing anesthesia. A T-tube was inserted into the trachea. (There was no increase in the dead space.) The $CO_2$ in the blood was determined by the method of Barcroft and Haldane; that in the alveolar air according to Henderson's method. (Inspiratory samples were taken.) The minute volume was determined by means of a Dreser tube for collecting expired air. We first did a series of controls and found that the $CO_2$ content of the blood and of the alveolar air varied with the minute volume, thus corroborating the work of Haldane and Priestley. Anesthesia produced effects varying only with the ventilation. In another series we inserted a valve which worked only one way and produced obstruction to expiration for varying lengths of time. This was our method of simulating the asthmatic attack. Control determinations were made before the use of the valve was begun. We found a marked increase in the $CO_2$ content of both the blood and the alveolar air, especially where compensation had not occurred. In most cases there was an attempt at compensation with regard to increased ventilation, due to the sensitiveness of the respiratory center to slight rises in the $CO_2$ pressure in the alveolar air. As soon as the obstruction was introduced, the type of breathing changed, expiration became long drawn out, the rate slowed, the abdominal wall muscles also taking part in the attempt to force air out. We thought therefore that increased muscular work might be a factor tending to raise the $CO_2$ content of the blood.

We therefore did a series of controls with strychninized dogs, and got results similar to those of our controls where increased ventilation was followed by a fall in blood and alveolar air $CO_2$ and vice versa. To get some insight as to what was happening in the circulatory system, blood pressure observations were made simultaneously with the determination of the intrabronchial pressure.

From a study of the tracings, we can say that there is at the beginning of expiration, a preliminary squeezing out of blood from the pulmonary capillaries and veins into the left heart. This increases the systolic output from the left ventricle and the carotid pressure therefore rises for a few seconds. After a few beats, however, there is a fall in blood pressure due to the fact that the increased intrabronchial pressure exceeds the capillary pressure, and interferes with the flow through the compressed capillaries thus diminishing the return to the left auricle. Suddenly at the beginning of inspiration, when the intrabronchial pressure falls, the drop in systolic blood pressure which began toward the end of expiration is still further increased for a few beats, the depleted pulmonary capillaries taking up the blood from the right ventricle and lessening the flow to the left heart.

There is therefore a distinct interference with blood flow through the lungs, and therefore with proper aeration. Gerhardt, Minkowski, Tendloo, Stewart and Romanoff are agreed that even slight rises in intrabronchial pressure cause considerable obstruction to blood flow through the lungs, the pressure in the pulmonary capillaries and veins being little above zero.

Hoover has attempted to explain the insufficient aeration of the blood in asthmatics on a respiratory basis. He found that the $CO_2$ content of alveolar air rose in the asthmatic attack. He first thought that an increase in the dead space, with impaired alveolar ventilation, was the cause of this rise. But later observations showed that there was no appreciable increase in the dead space in these cases. In fact he says "the dead space is no larger in these cases than in normal persons." The cause of the disturbance in aeration could not be circulatory, for he says "to produce cyanosis by impairment in circulation, evidence of stasis must be very great."

Yet we all know that most emphysematous patients show rather well marked cyanosis without any evidence of hepatic congestion, edema, etc. He concluded from his studies "that the real difficulty of ventilation in asthma and emphysema lies in a distention of the infundibula and this fails to allow an equal diffusion of $CO_2$ throughout the alveolar air." Yet he adds, "when we consider that an infundibulum has a diameter of not more than 1 mm. and that in hyperdistention the infundibula cannot be enlarged more than 2 to 3 mm., it seems astonishing that when 2 liters of air distributed in such small chambers are diluted with the addition of 3.5 liters the diffusion of gases is not uniform throughout the entire mass." To which we would add that one must remember that $CO_2$ has a coefficient of diffusion twenty times that of oxygen.

In view of these facts, and our own studies, we are inclined to believe that the circulatory factor, contrary to Hoover, is the important one. The cough in asthmatics, with its rises in intrabronchial pressure, would certainly embarrass the lesser circulation, and the voluntary attempts to aid expiration would act in the same manner. There is here a long-drawn-out expiration with a rise in intrabronchial pressure, as a result of which resistance to the flow through the pulmonary capillaries is great. During this phase there is little opportunity for gaseous exchange. Then there follows only a short inspiration during which the $CO_2$ is released as the blood is taken up by the depleted pulmonary sponge. The absence of a permanent fall in blood pressure in our animal may be explained by a compensatory vasomotor contraction of the systemic arterioles, and the rise in intra-abdominal pressure. From our studies we therefore conclude that the high $CO_2$ content of the alveolar air in asthma, and obstructed expiration in general is due to a circulatory cause. The rise in intrabronchial pressure during the long-drawn-out expiration interferes with the free flow through the pulmonary circuit, thus causing a damming back of the blood to the venous side. There is a consequent accumulation of $CO_2$ in the blood with the liberation of the alveolar air $CO_2$ chiefly during the short inspiratory phase of asthmatic breathing.[1]

[1] We might add that a pathologic study was made of the lungs of our valve dogs by Dr. Alexander Fraser, of the Department of Pathology of University and Bellevue

45 (1109)

## A height-weight formula to estimate the surface area of man.

By Delafield Du Bois and E. F. Du Bois.

[*From the Russell Sage Institute of Pathology in Affiliation with the Second Medical Division, Bellevue Hospital.*]

Using the method previously described before this society,[1] it has been possible, with the aid of Miss Sawyer and Mr. Stone, to measure the surface area of a total of 10 individuals. The "Linear Formula" previously described, when applied to these, gives an average error of 1.5 per cent. The chief limitation of the "Linear Formula" is that it involves the taking of 19 measurements. In the literature of the respiratory metabolism already published the only data given in regard to most of the subjects are the height and weight.

On the basis of the actual measurement of the surface of 10 subjects of widely varying shape, formulas have been constructed on the plan of $A = W^{1/a} \times H^{1/b} \times C$, in which $A$ is the surface area in square cm., $W$ is the weight in kilograms and $H$ the height in cm. and $C$ a constant. Various formulas were tried, care being taken that $3/a + 1/b$ should always equal 2 in order that the formula might remain bi-dimensional. The formula $A = W^{1/3} \times H^{1/1} \times C$ gave an average error of $\pm$ 3.3 per cent. The formula $A = W^{1/2} \times H^{1/2} \times C$ gave an average error of 2.2 per cent. It was evident that values for $1/a$ somewhere between 1/3 and 1/2 would give better results. By a rather lengthy process of calculation it was found that the average error could be reduced to 1.7 per cent. if the formula were made $A = W^{1/2.35} \times H^{1/1.38} \times C$. The calculation is not difficult if logarithms be used but a chart has been devised by means of which it is possible to estimate the surface area at a glance. The ordinates represent the height

Hospital Medical College, to whom we here express our sincere thanks. He found the macroscopic and microscopic evidences of emphysema, with the exception of increase in connective tissue. There were also areas resembling infarctions probably due to rhexis as a result of the increased blood pressure in the pulmonary circuit.

[1] D. Du Bois and E. F. Du Bois, Proc. Soc. Exp. Biol. and Med., 1914, XII, 16; Arch. Int. Med., 1915, XV, 868.

in cm., the abscissæ the weight in kilograms and curved lines drawn diagonally across the chart give the readings for the surface area in square meters. The details of the work with the chart and values of the constants will appear shortly in the Archives of Internal Medicine.

46 (1110)

**Diabetic dietetics. Glucose formation from protein foods.**

By **N. W. JANNEY** and **F. A. CSONKA** (by invitation.).

[*From the Chemical Laboratory of the Montefiore Home and Hospital, New York City.*]

The carbohydrate content of foods has been usually accepted as a gauge of their adaptability to the dietary of diabetics. It has, however, been demonstrated that proteins yield in the glycosuric organism large amounts of glucose (50 to 80 per cent. of their weight, Janney). An exact knowledge of how much sugar arises in diabetic metabolism from protein food is therefore of some importance.

By observing certain precautions it was found possible to determine rather accurately the amounts of glucose yielded by various meats fed to dogs made completely diabetic by phlorizin. The sugar formed from beef, chicken, chicken eggs, rabbit and fish could thus be ascertained to represent from 9 to 12 per cent. of the uncooked moist food. The solid substances of these materials produced from 36 to 48 per cent. glucose. Broiling and frying lead to considerable loss of water with corresponding increase of the percentage formation of glucose. Broiled beefsteak would yield 17.5 per cent. glucose.

Flour fed in preliminary experiments of the same nature gave rise to 92.5 per cent. of sugar on an anhydrous basis of calculation. From this data it was computed that in regard to sugar production or liberation in the organism 100 gm. bread is equivalent to about 350 gm. broiled beefsteak. In formulating diets for diabetics, glucose formation from the protein as well as the carbohydrate content of the food should therefore be considered. The experiments here alluded to will be later reported in detail.

47 (IIII)

## Effect of fatigue upon gastro-intestinal motility.

By LUDWIG KAST.

[*From the New York Post Graduate Medical School.*]

On fifty-four patients and healthy individuals tests were made regarding the motor power of the stomach and intestines by means of X-ray examinations, and the stomach tube. In addition carmine was used for the determination of time during which same was entirely eliminated from the body. The tested subjects were kept on a standard diet for a number of days and the motor efficiency of the stomach and intestines was determined repeatedly by these methods. By taking the average, it was determined how long it took the stomach, and separately, how long it took the intestines, to propel the given amount of food. During the period of tests, the amount of physical activity was regulated and the mental activity approximately limited. Without any change of diet and mental activity, the physical activity was markedly increased or decreased and the effect of same upon the evacuating power of the stomach or intestines observed. At other times, without change in the diet or the amount of physical exercise, the amount of mental efforts was markedly increased or decreased and the effect observed in the same manner. Summarizing the results of such experiments, it appears that the healthy individual showed very little variation as regards the evacuating power of the stomach or intestines as long as the physical exercise was not excessive. Mental efforts had no perceptible effect.

In patients with moderate and marked degrees of atony and splanchnoptosis, the evacuating power of the stomach and intestines decreased in direct proportion to the amount of mental and physical exertion. Physical exertion had the same effect in such individuals if the patient was kept in a horizontal position during these exercises and during the period of observation in order to eliminate the effect of splanchnoptosis in the erect position. In patients who clinically appeared susceptible to the effects of fatigue, mental exertion was more marked in its delaying effect than physical exertion.

Recognizing the difficulty of even approximate measurements of physical and mental efforts during several days, it is evident that only a large number of similar experiments are apt to reduce the effect of accidental factors or of physiological fluctuations in the motor efficiency of the gastro-intestinal tract.

48 (1112)

## On the behavior of the mammalian ovary and especially of the atretic follicle towards vital stains of the acid azo group.

By HERBERT M. EVANS.

[*From the University of California, Berkeley, California.*]

I have given elsewhere[1] a description of those cells of the mammalian body which react so predominantly even if not in a wholly specific way with vital dyes of the acid azo series as to justify their recognition as a great functional unit or cell class. For the cells in question it is suggested that we retain the old term macrophage, which although proposed by Metchnikoff without the kind or the complete extent of evidence now available for delimiting the class, nevertheless puts in the foreground their salient structural and functional peculiarity and has the further advantage of enabling us to coördinate these studies with those long made by the comparative pathologist.

It is worthy of note that in some of those cases of local tissue degeneration and death which one must regard as physiological or normal, the macrophages must, in analogy with the experience of pathologists, be actively concerned. This above all is exemplified by the cyclic changes undergone by the mammalian ovary. The strange cells which since the time of Pflüger have been known to be of assistance in atresia of the follicle and whose derivation from granulosa or theca or from leucocytes, *i. e.*, from practically every available source, has in turn been championed—are picked out by the azo dyes so brilliantly and so electively as to preclude the denial of their alignment as typical macrophage cells

There will be demonstrated a series of drawings of these colonies of macrophages in the atretic follicle of the mouse, rat,

[1] Evans, H. M., "The Macrophages of Mammals," *Am. Jour. Physiol.*, Vol. 37, No. 2, May, 1913.

guinea pig, rabbit, dog and monkey, in the latter of which the conditions are so similar as to stand for the case in man.

Striking as they are, these studies are not sufficiently indicative of the altogether unusual affinity of the atretic ovum macrophages for these dyes, a fact which forces itself on our attention when small doses of the dyes are given. The preparations showing the scanty macrophage content of the peculiar atresia of the dog demonstrate also that although but little general staining resulted, these cells have all accumulated dense deposits of the vital dye.

The macrophages are the cells which penetrate the zona pellucida of the degenerate ovum and in late stages of atresia may be present solely within the zona.

A different and more unique reaction in the ovarian follicle must now be mentioned. Impending atresia in the good-sized follicle has as its ear-marks a reaction never seen in the healthy state, for before the nuclear disorganization seen by Fleming and Schottander takes place the granulosa cells destined to perish have suddenly become permeable to the vital stain which they house in cytoplasmic granules frequent enough to mark out the whole layer as deeply stained. On the downhill, as it were, these cells never increase in size or function so as to often be confused with the macrophages. This reaction of the granulosa is significant one must feel, chiefly as proof not only of preliminary cytoplasmic as against nuclear change but of physical change in the protoplasmic state. It will be well now to know whether the differing behavior of the granulosa cells is dependent on a changed protoplasm into which now diffusion can take place (increased permeability) or whether it be due essentially to electrical surface changes which let adsorptive forces operate. The conditions which bring about this reaction are typically seen in the atresia which always overtakes the next succeeding crop of Graafian follicles after fertilization of the preceding crop, but this behavior is not repeated further in the pregnancy, where now other forms of atresia may come in. Enough warrant consequently exists for the recognition of types of atresia the occurrence of which is related with certainty to what one may broadly term the cycles undergone by the ovary in general. An examination of this point in animals where with many individuals we have followed the sexual cycles, is in progress.

# SCIENTIFIC PROCEEDINGS

## Abstracts of Communications.

### Seventy-third meeting.

*College of the City of New York, February 16, 1916.*
*President Lusk in the chair.*

49 (1113)

### Concerning the protein content of meat.

By **N. W. Janney.**

[*From the Chemical Laboratory of the Montefiore Home and Hospital for Chronic Invalids, New York.*]

The commonly accepted modes of determining the protein content of animal muscle are open to criticism. Thus in such standard works as that of König,[1] also in Atwater and Bryant's[2] extensively quoted tables, the protein material has been usually calculated by multiplying the total nitrogen content of the fresh meat in per cent. by the factor 6.25. This, as is known, introduces a considerable source of error, for of the total nitrogen about 13 per cent. is combined in non-protein substances. Moreover the factor 6.25 is of itself incorrect. It is obtained on the basis of accepting 16 per cent. as the average nitrogen content of meat proteins, whereas it has been recently established in this laboratory that the correct value lies between 16.2 and 16.7 per cent. of the pure muscle proteins of various species.

A second indirect method of calculating the "protein substances" of meat has also been recognized by Atwater and Bryant. According to this procedure the combined weights of the ether

[1] König, J., "Chemie der Menschlichen Nahrungs und Genussmittel," Berlin, 1903.

[2] Atwater, W. O. and Bryant, A. P., U. S. Dept. of Agriculture, Bull. 28 (rev.), 1906.

soluble substances plus the ash is deducted from the total solids of the meat and the result considered as representing protein substance. Likewise this scheme falls short of an accurate determination of the muscle proteins. Indeed even the total nitrogenous substances cannot be thus obtained with precision, for ether removes from meat various bodies containing nitrogen.

The cause of these difficulties and uncertainty has been the lack of an accurate analytical procedure for the direct determination of the proteins in muscle. It was however found practicable to develop such a method,[1] an improved form of which will appear elsewhere. The principle involved in the modified procedure is coagulation of the muscle in alcohol and the removal of the non-protein material by extraction. When this is carried out with certain precautions it is possible to completely separate the fatty and other non-protein material from the proteins, which can be obtained in a high state of purity.

The results thus arrived at by direct analysis are compared in the following table to those obtained by calculation as above indicated. The computed values exceed that of the protein actually present, by fifteen to twenty per cent. In dietetic and metabolic studies this discrepancy must therefore be considered. A revision of various tables of food analyses in this respect is indicated.

PROTEIN CONTENT OF MUSCLE.

| Species. | Total Nitrogen in Muscle. | Protein Calculated (Total N × 6.25). | Protein Actually Present. | Nitrogen[2] in Protein. |
|---|---|---|---|---|
| Dog | 3.33 | 20.8 | 17.9 | 16.3 |
| Rabbit | 3.39 | 20.8 | 16.7 | 16.3 |
| Chicken | 3.43 | 21.4 | 17.5 | 16.4 |
| Fish (halibut) | 3.10 | 19.4 | 16.7 | 16.4 |

[1] Janney, N. W., and Csonka, F. A., *Jour. Biol. Chem.*, 1915, XXII, 195.

[2] Not calculated ash free.

50 (1114)

## The epinephric content of the blood in conditions of low blood pressure and "shock."

By **E. A. BEDFORD** and **H. C. JACKSON.**

[*From the Department of Physiology, University and Bellevue Hospital Medical College.*]

The following is a brief record of experiments undertaken to determine whether in low blood pressure there is an increased activity of the adrenals.

Dr. Alfred R. Allen, who presented before this society the results of an experimental study of the condition of the Purkinje cells in low blood pressure and shock, stated in conversation with the authors that he found marked histological changes in the adrenals under similar conditions. In the present series of experiments an attempt has been made to answer the question both qualitatively and within limits, quantitatively by an examination of the blood of animals (dogs) taken from the adrenal vein, before and after a more or less prolonged condition of low blood pressure.

In order that blood from the adrenal might be obtained undiluted by the general venous blood, all veins, except the inferior mesenteric and the left adrenal, entering the vena cava for some distance on either side of the adrenal vein were ligatured. Through the inferior mesenteric a canula was inserted into the vena cava, in such a way that the flow of blood in the vena cava was unobstructed. At the time of drawing blood, the vena cava above and below the entrance of the adrenal vein was closed by clamps, having rubber protected jaws. The first blood, that in the enclosed segment of the vena cava, was discarded.

Care was taken to measure the rate of flow in order to eliminate the possibility that results obtained might be due to a greater concentration of epinephrin, because of a less rapid flow of blood through the organ, although its activity might not be increased. At the beginning of the experiment, blood to be used as control was taken from the jugular vein.

Carotid blood pressure was taken.

For the determination of epinephrin in the blood, an adapta-

tion of Hoskins's method was used. The essential part of the method is that the tone of rabbit intestine, immersed in oxygenated blood at 37 degrees C., is lowered and the rhythmic contractions more or less inhibited by the presence of epinephrin in the blood.

To determine the quantitative relation of epinephrin in the samples of blood tested, two methods were used. In one method, the tracings obtained were compared with tracings obtained on the addition of known amounts of adrenalin to control blood. In the second method, the blood giving the reaction for epinephrin was diluted with control blood until the reaction of this blood was similar to that of the compared blood.

Low blood pressures were brought about by one of there methods;

1. Handling of intestines.
2. Hemorrhage.
3. Occlusion of the thoracic inferior vena cava.

In most of the experiments, pressures of 30 to 40 mm. of mercury were obtained.

In all three types of experiments, the epinephric content of the adrenal blood was increased, provided that the pressure was sufficiently low and the condition of low pressure was maintained for a sufficient length of time. Since the blood was diluted with control blood to compensate for the difference in the rate of flow through the adrenal organ, an increased activity of these organs was indicated.

In some cases, it was necessary to dilute the experimental adrenal blood with thirty-two times its volume of jugular blood, before a tracing could be obtained similar to that of adrenal blood, drawn before low pressure was induced. In other cases the reaction was similar to the reaction given by control blood to which had been added adrenalin sufficient to make a 1 to 10,000,000 dilution.

In experiments in which samples were taken at intervals, it was shown that the marked increase of epinephric content of blood occurred only after a considerable duration of a condition of low blood pressure, varying from one to two hours.

In these experiments, the later samples indicated an increasing amount of epinephrin in the blood.

Most of the experiments in which pressure was not permitted to go below 50 or 60 mm. Hg gave negative results. A few of the handling experiments were exceptions.

These negative experiments served as controls, indicating that the anesthetizing and general operative procedure did not bring about the results obtained.

To be certain that the results were due to the presence in the blood of the secretion of the adrenal gland and not to the secretion of some other organ, for example the pituitary body, the adrenals were ligatured in such a way that while the blood from the lumbar branch of the adrenal vein was permitted to enter the vena cava, no material could pass from the adrenal organ into the circulation.

Only negative results were obtained under these circumstances.

These experiments, therefore, seem to indicate that an increased activity of the adrenals accompanies a somewhat prolonged low blood pressure condition.

## 51 (1115)

## On the augmenting action of ergotoxine (Dale and Barger) on the gastrointestinal movements.

By **S. J. MELTZER** and **T. S. GITHENS**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

About ten years ago Meltzer and Auer[1] reported animal experiments in which intravenous injection of ergot augmented strongly the spontaneous movements of the gastrointestinal canal and increased the motor responsiveness of the canal to vagus stimulation. In these experiments a fluid extract of ergot (U. S. P.) was used. At about the same time Dale and Barger succeeded in isolating from ergot an alkaloid which they named ergotoxine. In their interesting publication on that preparation a year later they ascribed the characteristic physiological effects of ergot to the presence of this alkaloid. With reference to the action upon the gastrointestinal movements they emphatically state that the effect is comparatively slight and inconstant, and believe that

[1] *Amer. Jour. of Physiol.*, XVII, 143, 1906.

the augmentation of the movements of the intestines observed by Meltzer and Auer must not have been due to a principle peculiar to ergot. "The effect on the intestinal movements," they state, "of a complex fluid such as the liquid extract, containing, apart from principles the action of which is peculiar to ergot, choline and various other vascular depressants (ergotoxinic acid, etc.), seems to us to need a more critical analysis before any great importance is attached to it as a specific action."[1]

On account of that statement the behavior of peristalsis was studied by us under the influence of Dale and Barger's specific alkaloid of ergot, ergotoxine.[2] We shall confine our present communication to the results which we have obtained in the experiments on rabbits. The gastrointestinal gut was observed in a trough made by suspension of the incised abdominal wall and kept filled with a warm Ringer solution. The animals received artificial respiration during the entire experimental observation. The results were unmistakable and easily demonstrable. Against Dale and Barger we must insist that *augmenting action of their ergotoxine upon peristalsis is very pronounced and constant.* It is only indispensable that the animal should not be too deep under the influence of ether, the only narcotic which we have used in the present experiments. A trick which favors further the augmenting action of ergotoxine upon peristalsis is the injection of a warm isotonic solution (0.9) of NaCl into the fundus of the stomach. After intravenous injections of 10 mgr. of ergotoxine, not only the pendular movements and the circular constrictions become greatly intensified, but the contents of the intestines are seen carried down by "peristaltic rush" (Meltzer and Auer)[3] through large parts of the small intestines and even through their entire length from the stomach to the cecum. The movements are followed by a strong constriction of the intestine extending over an inch and longer. Even the empty parts of the intestines of a ribbon-like relaxed appearance show unmistakable contractions after an injection of ergotoxine. The

[1] *Biochemical Journal*, II, 287, 1907.

[2] It was obtained from Burroughs, Wellcome & Company. The alkaloid is prepared in the Wellcome Physiological Research Laboratories, London, of which H. H. Dale is the director.

[3] *Amer. Jour. of Physiol.*, XX, 259, 1907.

peristaltic augmentation became manifest also in parts of the colon and not infrequently even in the otherwise inert cecum. The augmented waves of the stomach are not very pronounced but the pyloric part of the stomach often contracts strongly as a whole. The vagus nerves were stimulated within the thorax in their course upon the lower part of the esophagus. Ergotoxine unmistakably increases the motor responsiveness of all parts of the gut to stimulation of the nerves even when their cardiac action is in no way involved.

52 (1116)

## An allergic skin reaction to diphtheria bacilli.

By J. A. Kolmer, M.D.

[*From the McManes Laboratory of Experimental Pathology of the University of Pennsylvania and the Laboratory of the Philadelphia Hospital for Contagious Diseases.*]

While immunity in diphtheria may be regarded as being principally antitoxic in nature, it is highly probable that antibodies of a lytic nature may be concerned. With this in view, we have applied an allergic skin reaction in addition to the toxin test of Schick, in studying immunity in diphtheria to the following persons:

1. To 123 persons of various ages, most of whom were healthy and well and had never had diphtheria or received an injection of diphtheria antitoxin.

2. To 61 persons receiving curative or prophylactic doses of diphtheria antitoxin.

The antigen for the allergic tests was prepared of 45 recently isolated cultures of diphtheria bacilli of various types; each culture was grown in glucose broth for four days and all mixed in a single flask and shaken mechanically with glass beads to break up clumps. To each 100 c.c. of the emulsion was added 5 c.c. of sterile horse serum antitoxin (2,500 units) and the whole shaken at room temperature for four hours. After this time the emulsion was placed in sterile centrifuge tubes and the bacilli separated and

washed twice with large volumes of sterile salt solution. After the final washing the bacilli were re-suspended in sufficient sterile salt solution to make, after thorough shaking, about two billion bacilli per cubic centimeter. This emulsion was heated at 60° C. for an hour; cultured for sterility and preserved with 0.2 per cent. tricresol. Subcutaneous injection of 1 and 2 cubic centimeters into 250 gram guinea-pigs showed absolutely no evidences of local reaction or general toxemia. In conducting the test, 0.1 c.c. of the emulsion which we have called *diphtherin*, was injected intracutaneously in the arm.

Reactions with the *diphtherin* were usually well marked and of two types, papular and pustular reactions. The latter were more severe than the former and both occurred with well-defined zones of erythema. These reactions usually reached their height within seventy-two hours and then began to recede.

The toxin tests were conducted with one-fortieth the M. L. D. of toxin diluted with sufficient normal salt solution containing 0.2 per cent. tricresol to render the dose 0.1 c.c. which amount was injected intracutaneously.

The throats and noses of a large number of persons were cultured to study the relation between the occurrence of positive reactions and the presence or absence of diphtheria bacilli in the upper air passages.

The bacteriolytic power of the sera of persons reacting positively and negatively to the *diphtherin* test, for living diphtheria bacilli were conducted toward throwing more light upon the nature of the allergic antibody. Complement fixation and agglutination tests were likewise conducted.

The following is a summary of the results of this investigation:

1. An allergic skin reaction was observed in about 70 per cent. of children and 35 per cent. of adults following the intracutaneous injection of a polyvalent antigen of washed, neutralized and heat-killed diphtheria bacilli.

2. These reactions were regarded as allergic in character and therefore entirely distinct from the toxin reaction of Schick.

3. About 53 per cent. of persons of various ages yielded positive *diphtherin* and negative toxin (Schick) reactions. About 10 per cent. yielded negative *diphtherin* and positive toxin reactions,

both tests agreeing therefore in about 63 per cent. of persons; 12.5 per cent. reacted positively and 24.1 per cent. negatively to both tests.

If a positive *diphtherin* reaction may be regarded as an index of lytic immunity, only 10 per cent. of persons were found who did not show the presence of either an antitoxic or lytic immunity, while 53.3 per cent. showed both types of antibodies; 24.1 per cent. showed antitoxic immunity only and 12.5 per cent. allergic, but no antitoxic antibody.

4. The percentage of positive *diphtherin* reactions was slightly greater among those who were convalescent from diphtheria.

5. There is no relation between the occurrence of positive and negative *diphtherin* and toxin reactions and the presence or absence of diphtheria bacilli; a negative toxin reaction in a person presenting clinical evidences of infection indicates that the individual does not require antitoxin but nothing more; he may be infected with virulent diphtheria bacilli capable of disseminating the disease.

6. The sera of persons yielding positive *diphtherin* reactions were not found to possess demonstrable bacteriolytic properties for diphtheria bacilli.

7. The sera of persons yielding positive *diphtherin* reactions yielded weakly positive or negative complement fixation and agglutination reactions with *diphtherin* as antigen.

8. Whether or not the *diphtherin* reaction will prove of practical value in handling outbreaks of diphtheria from the standpoint of passive immunization and diagnosis will depend upon future experiences under such conditions and also upon the results of experimental work bearing upon the broad question of allergic reactions as an index of immunity; it would appear at least that more attention should be paid the question of bacteriolytic immunity in diphtheria.

53 (1117)

## Nitrogen retention in nephritis in children.

By IRVING S. CUTTER and MAX MORSE.

[*From the Biochemical Laboratory, University of Nebraska, College of Medicine, Omaha.*]

The writers have studied the urinary excretion of nitrogenous products in nephritic children and conclude that retention, such as is described for all of these components in adults by Mosenthal, Folin, Foster and others for cases of nephritis does not obtain in children. It is to be expected that as far as some of the components are concerned, such as creatin, the adult and child condition would be different for the metabolism of creatin in children differs from that of the adult. We have found that retention of creatin and creatinin is a matter of twenty-four hours or less and that no retention occurs beyond that time in the cases examined. The excretion of these constituents was followed by studying 24-hour specimens, supplemented by shorter time specimens and temperature, food, etc., were checked in all cases. In none of the cases studied were the creatin results vitiated by the acetone nor by the aceto-acetic acid factor, attention to which was duly paid. The figures obtained from the nephritic children were lower than those obtained from a study of a number of normal children from the Child Saving Institute, but this is not interpreted as meaning retention, for immobility in children involves lower nitrogen output in general. The marked divergence in data of creatinin excretion in the nephritic children from day to day is typically different from the figures obtained by others in the adult, for the typical condition in the adult is constancy in amount of excretion, whereas in the children the variation from day to day was marked. The writers are not aware of a similar study having been made previous to the present one.

54 (1118)

## The effect of moderately high atmospheric temperatures upon the formation of hemolysins.

By C.-E. A. Winslow, James Alexander Miller and W. C. Noble.

[*From the New York State Commission on Ventilation.*]

The experiments which have been reported in regard to the effect of high atmospheric temperatures upon susceptibility to bacterial infections, or upon the immunity reactions in response thereto, seem at first sight to be conflicting and unsatisfactory. Some authors report increased resistance as a result of external heat and others precisely the reverse. A more careful analysis shows however that if the several factors at work in such experiments and the various conditions employed by different investigators be considered, the results are reasonably harmonious. A moderate amount of heat may naturally be expected to produce a different result from temperatures so severe as to lead to a condition of fever in the experimental animals; and exposure to a hot atmosphere may produce one effect on the susceptibility of an animal to subsequent infection and quite another on the course of an infection already established.

The majority of investigators have been chiefly interested in the effect of the condition of fever upon recovery from infection, and have therefore exposed their animals to atmospheric conditions sufficiently extreme materially to increase the body temperature. Experiments of this kind have quite uniformly indicated that the progress of an infection already established is in greater or less degree checked by an artificial fever due to a very high atmospheric temperature, or produced by the Sachs-Aronson operation. Such experiments have been made and such a conclusion reached by Rovighi, Walther, Filehne, Hildebrandt, Loewy and Richter, Kast, Engelhardt, and Rolly and Meltzer. In all these experiments the high atmospheric temperatures used were 35°–41° C. and the body temperatures of the animals 40°–42°. Vincent and

Sacquépée and Loiseleur on the other hand found resistance lowered by high heating, but for the most part their experiments were concerned with the lighting up of latent infection or the invasion of bacteria from the digestive tract, a very different phenomenon from the progress of the struggle for immunity against an infection already established.

Finally there is another type of experiments in which the effects upon vital resistance of a moderately high temperature (30°–35°) have been studied; and these experiments yield results quite different from those which have just been reviewed. While a temperature approaching 40° by producing a state of fever appears to favor recovery from an infectious disease, a somewhat lower temperature seems to exert a lowering effect on general vital resistance without the compensating stimulation of vital processes which may accompany the development of fever. Five different investigations, the only ones with which we are familiar bearing on this point, all warrant the same conclusion. Fermi and Salsano (1892) found that a strain of avian tubercle bacilli which was incapable of producing a general infection in normal guinea pigs could be found in abundance in the glands of animals kept at 33°–35°. Similarly mice when heated showed many more tubercle bacilli, of both avian and human types, in their glands than did control animals; and the infection was still further increased by combining high humidity with the high temperature. Graziani (1906) studied the agglutinating power of the blood of rabbits kept at various temperatures. At 2° to 4° the blood would agglutinate at a dilution of 1 in 1541; at 18°, 1 in 854; at 32°, 1 in 727. In another series the blood of rabbits kept at 32° agglutinated at a dilution of 1 in 1250 while if the animals were occasionally relieved by cold baths the agglutinating power rose to 1 in 2425. Ritzmann (1907) kept guinea pigs, white rats and mice at 35° and found that heated animals died from half a day to three and a half days after injections of streptococci, control animals after one and a half to eight days. Injections of toxin-free tetanus spores and of tetanus spores plus streptococci yielded similar results. Ritzmann also cites experiments of Wysso-kowitsch leading to the same conclusion. Finally Ruata (1909) kept guinea pigs at a temperature of 30° with a relative humidity

of 85–95 per cent. and injected them with doses of typhoid, paratyphoid, dysentery and colon bacilli and cholera spirilla which were not fatal for normal animals. All the guinea pigs thus treated died in 4–26 hours, while, of control animals exposed to the heat alone, without injections, 30 per cent. succumbed.

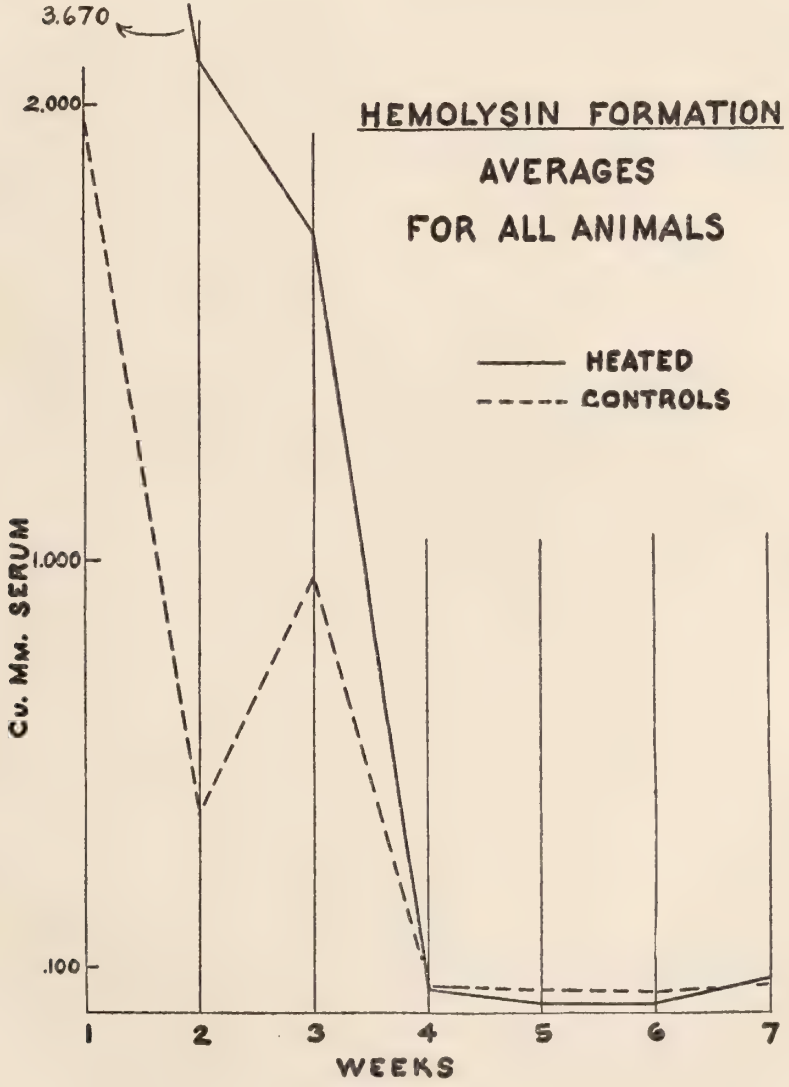


FIG. 1.

Our own experiments, which were undertaken as a part of the extensive studies of the New York State Commission on Ventilation, have dealt with this same problem of the effect of moderately high temperatures and were carried out in the bacteriological laboratores of the University and Bellevue Hospital Medical College.

Normal healthy rabbits were kept (2–4 at a time) in an incubator, 25″ x 48″ x 12′, at a temperature ranging from 29°–32° C. Control animals were kept at room temperature (18°–21°). At the beginning of the experiment each rabbit was bled (1 to 2 c.c.) and then inoculated intravenously with ½ c.c. of a 50 per cent. suspension of washed sheep erythrocytes. During the experimental period each rabbit was bled once a week for trial titrations of the hemolytic activity of the serum, and inoculations with the sheep cells in increasing doses were made twice a week during this period.

The hemolytic activity of the serum was determined as follows: The rabbit serum was inactivated at 55° C. for one half hour. A 5 per cent. suspension of sheep corpuscles was used, and for complement, normal guinea pig serum diluted 1–10.

The rabbit serum was prepared in varying dilutions, as indicated by the results of the previous titrations, and each dilution was then titrated in the same way.

A series of ten test tubes was set up with 0.1 c.c. of sheep corpuscle suspension and 0.1 c.c. of diluted guinea pig complement, and varying amounts of rabbit serum. The test tubes were then placed in a water bath at 37° for 1 hour. At the end of that time readings were made, and the smallest amount of rabbit serum of the dilution which gave complete hemolysis was taken as the hemolytic unit. Thus, if .06 c.c. of a dilution of 1–500 was the smallest amount of serum giving complete hemolysis, then .06 of this solution was taken as the hemolytic unit (.06 x 1/500 = 1/8333 c.c. = .120 cubic millimeters) and this decimal, representing the actual dilution of serum in cubic millimeters found effective under the conditions of the experiment, was taken as the measure of the hemolytic power of the serum. The figures in the table (used as ordinates in the chart) are derived in this way. 10 in the table means that 1/100 of a c.c. of serum (10 cubic mm.) showed no hemolytic activity. The error introduced into the

calculation of averages by calling this figure 10 when we only know that it was greater than 10 will not materially affect the results. Titrations were not performed during the second week of Series II. Series I and III were stopped after six weeks, Series V after five weeks and Series IV after four weeks. Other blanks in the table are due to the death of the animals.

| Series. | Rabbit. | Air Temperature. | Hemolytic Power of Serum. (Cubic Millimeters of Serum Necessary to Hemolyze.) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 Week. | 2 Weeks. | 3 Weeks. | 4 Weeks. | 5 Weeks. | 6 Weeks. | 7 Weeks. |
| I | 1 | 30° | 10.000 | .118 | .070 | .. | .. | .. | |
| | 2 | 30° | .500 | .083 | .070 | .059 | .044 | .022 | |
| | 51 | 20° | 10.000 | .161 | .073 | .. | .. | .. | |
| | 52 | 20° | 10.000 | .069 | .050 | .060 | .105 | .089 | |
| II | 186 | 30° | .145 | .. | .069 | .020 | .040 | .047 | .067 |
| | 187 | 30° | .100 | .. | .036 | .034 | .032 | .054 | .084 |
| | 183 | 20° | .178 | .. | .075 | .014 | .040 | .055 | .067 |
| | 184 | 20° | .189 | .. | .024 | .040 | .020 | .033 | .061 |
| III | 70 | 30° | .588 | .060 | .028 | .025 | .029 | .033 | |
| | 75 | 30° | 1.000 | .067 | .042 | .022 | .033 | .040 | |
| | 86 | 20° | .213 | .050 | .024 | .020 | .025 | .029 | |
| | 110 | 20° | .500 | .067 | .029 | .017 | .022 | .028 | |
| IV | 88 | 30° | .588 | .075 | .020 | .026 | | | |
| | 171 | 30° | .588 | .069 | .025 | .032 | | | |
| | 4 | 20° | .500 | .027 | .020 | .026 | | | |
| | 191 | 20° | .200 | .044 | .015 | .020 | | | |
| V | 72 | 30° | 10.000 | 10.000 | .172 | .. | .. | | |
| | 77 | 30° | 10.000 | .100 | .164 | .. | .. | | |
| | 100 | 30° | 10.000 | 10.000 | 10.000 | .200 | .044 | | |
| | 173 | 30° | .588 | .400 | 10.000 | .063 | .044 | | |
| | 83 | 20° | .123 | .062 | .. | .. | .. | | |
| | 136 | 20° | .238 | .270 | 10.000 | .238 | .083 | | |
| | 147 | 20° | .833 | .097 | .076 | .110 | .083 | | |
| | 148 | 20° | .588 | .588 | .161 | .047 | .040 | | |
| General average . | | 30° | 3.670 | 2.100 | 1.720 | .053 | .038 | .039 | .076 |
| | | 20° | 1.970 | .144 | .958 | .059 | .052 | .047 | .064 |

The results as presented in the table appear to indicate a distinct decrease in the rate of hemolysin formation on the part of the heated rabbits. The hemolytic power of the blood of individual animals of course varies within wide limits, yet the averages show that in order to produce hemolysis it was uniformly necessary to use larger quantities of serum from the heated rabbits during the first three weeks. The influence of heat appears to show itself in a delayed formation of hemolysins rather than in a

permanent inhibition, as later on the average curves for heated and control animals are essentially the same. In Series III there were only two occasions in which the lowest serum strength for a heated rabbit fell below the highest for a control rabbit; and in Series IV, not one.

The wide variations exhibited in individual animals preclude the possibility of drawing definite and final conclusions from these results, but their general tendency, as evidenced by averages, agrees with the results of the other observers cited and they strongly suggest that moderately high air temperatures (30°) do not favor the development of immune bodies in the blood as higher temperatures producing a condition of fever have been reported to do, but on the other hand may be distinctly inimical to such development.

# SCIENTIFIC PROCEEDINGS

Abstracts of Communications.

**Seventy-fourth meeting.**

*Presbyterian Hospital, March 15, 1916.*

*President Loeb in the chair.*

55 (1119)

## The cytology of the exudate in the early stages of experimental pneumonia.

By **Frank A. Evans** (by invitation.).

[*From the Department of Pathology of the Presbyterian Hospital.*]

The cells have been studied in the early exudate of pneumonia produced in rabbits by intrabronchial injection of pneumococcus group I, group IV, and by an attenuated strain of pneumococcus furnished by Dr. Carrol G. Bull's laboratory at Rockefeller Institute; by streptococcus hemolyticus and by a streptococcus isolated from the mouth of a normal individual by Miss Olmstead of Presbyterian Hospital; and in the exudate in reaction to intrabronchial injection of 33 per cent. egg yolk in neutral broth. The early exudate in three cases of human pneumococcus pneumonia has also been available for study.

In all of these lesions, although many polymorphonuclears were often present, in many of the alveoli the cytology of the exudate was predominantly mononuclear in character. These mononuclear cells may be classified as follows: a few typical small lymphocytes of the blood; a few epithelial cells from the alveolar walls; relatively many oxydase-containing large mononuclears greatly resembling the so-called transitional cells of the blood; and almost as many non-oxydase containing large mononuclears of

the blood or closely related forms. In pneumonia induced in animals heavily stained with lithium carmine, no cells stained with carmine took part in the formation of the exudate. No plasma cells were seen.

56 (1120)

## Technique of cultivating human tissues in vitro.

By R. A. LAMBERT, M.D.

[*From the Pathological Laboratory of the Presbyterian Hospital.*]

Several difficulties have been encountered in the cultivation of human tissue in vitro.

In the first place human fibrin is readily liquefied by fresh tissue, so that when human plasma is used as a culture medium the cells find no framework on which to grow. Losee and Ebeling overcame this difficulty by transferring the tissue fragments at frequent intervals before liquefaction took place. We have solved the problem in another way which does not necessitate frequent transfers. The method consists in using as a culture medium chick plasma, the fibrin of which resists digestion, with the addition of an equal quantity or more of human serum. In this medium the cells grow much more actively than in pure chick plasma. Since there is no liquefaction it is not necessary to make subcultures oftener than every 5 to 7 days.

That fresh human tissue cannot always be obtained when wanted has appeared to be another difficulty in the study of human tissues in cultures. We have found, however, that human tissues, just as those of lower animals, may be preserved for 5 to 10 days before using, if cut into small pieces, covered with salt solution and put aside in a cool place. Serum and Ringer's solution possess no advantage over ordinary salt solution and a temperature of 15° C. appears to be as satisfactory as a lower temperature. Tissues obtained at autopsy may be used though often infected. We have obtained good growth of connective tissue from pieces of liver and testis taken from a body six hours after death.

The sterilization of infected tissues constitutes a problem which we have not yet solved satisfactorily. Skin, which is

practically always infected superficially, may be partially sterilized with little injury to the tissue by rinsing the surface quickly with weak alcohol (60 per cent.). In a large number of preparations from a piece of skin treated in this way, a fair percentage will show no bacterial contamination, and some of the remainder will show only occasional colonies. We have obtained a good growth of epithelium from pieces of circumcision tissue thus treated.

A large number of antiseptics and disinfectants—toluol, chloretone, tricresol, phenol, silver nitrate, hypochlorites (Dakin's solution), argyrol, iodine, potassium cyanide, and bichloride of mercury, have been tested on tissues more diffusely infected. For nearly all of these the strength of solution necessary to kill bacteria (staphylococcus aureus) also injures the cells.

Experiments carried out so far, however, indicate that potassium cyanide and probably also bichloride of mercury are exceptions to this rule. For example, potassium cyanide in 1–2,000 dilution is a very good disinfectant but injures cells very slightly. More complete reports of these experiments will be presented in a subsequent communication.

57 (1121)

## Development of immune reactions in serum disease.

By W. T. LONGCOPE and F. M. RACKEMANN.

[*From the Medical Clinic of the Presbyterian Hospital, Columbia University, New York.*]

The occurrence of immune reactions to horse serum and their relationship to the development of serum disease in man, we have studied by two methods: first, the sensitiveness of the skin to intravenous injections of 0.02 c.c. of horse serum, undiluted or diluted ten times or one hundred times with 0.85 per cent. NaCl; and secondly, by determining the presence of anaphylactic antibody in the blood serum of the patient by transference to guinea-pigs through passive sensitization.

Eleven patients have been studied, who have received for therapeutic purposes from 4 c.c. to 350 c.c. of horse serum, in the

form of diphtheria antitoxin, antimeningococcus serum or antipneumococcus serum, intravenously, intraspinally or intramuscularly. Nine of the eleven cases developed serum sickness.

All of the cases, whether or not they developed serum disease, showed sooner or later a positive specific reaction to the intracutaneous injection of horse serum. This was never obtained before the seventh day following the first therapeutic injection of horse serum and was first observed between this day and the eighteenth. It was never demonstrable until after the appearance of serum disease.

Anaphylactic antibodies could not be demonstrated in the two cases that did not develop serum disease. In all of the other nine cases these antibodies were found at some time in the serum of the patient. In but one case did they appear before the onset of serum disease and then on the fifth day after the therapeutic injection of horse serum. Neither in this instance nor in any other was the anaphylactic antibody demonstrable in the patient's serum during the early part of serum sickness. In all nine cases the anaphylactic antibody was present in maximum concentration at the close of the serum sickness and in one instance persisted for sixty-eight days after the disease. In two cases in which the original attack of serum sickness was followed by a relapse, the antibodies could not be definitely demonstrated until the end of the relapse, that is twenty-one and twenty-four days after the therapeutic injection of horse serum. In several instances it was possible to sensitize guinea-pigs both passively and actively to horse serum with portions of the same specimen of blood serum drawn from the patients towards the close of the serum sickness, thus demonstrating that some of the proteins of horse serum and antibodies for the proteins of horse serum may exist at the same time in the circulation in man.

These experiments show that anaphylactic antibodies for horse serum appear in maximum concentration in the blood serum towards the close of serum sickness and suggest that their presence in the circulation in large amounts determines the recovery from this disease.

58 (1122)

## Immunization with sensitized bacteria.

By HOMER F. SWIFT and RALPH A. KINSELLA.

[*From the Medical Clinic of the Presbyterian Hospital.*]

The object of the present study was to determine the relative immunizing property of various preparations of green streptococci. Two different strains of green-forming streptococci were used, both isolated from cases of acute rheumatic fever. The lethal dose of these organisms for mice, was from 0.1 to 0.5 c.c. of a twenty-four hours broth culture. The sensitized vaccine was prepared from a twenty-four hours broth culture, centrifugalized, washed, killed at 56°, strong anti-serum added, incubated one hour, washed and suspended in saline. Sensitized vaccines were always freshly prepared. Three different antibodies have been studied, agglutinins, complement fixing bodies and protective antibodies. Rabbits were immunized by first injecting dead organisms, later by living organisms and the comparative curve of antibody formation studied. With unsensitized vaccine there was strong formation of antibodies in from twelve to sixteen days, the curve for agglutinins, complement-fixing antibodies and protective antibodies running parallel. The animals immunized or rather injected with sensitized vaccines showed at times a late formation of weak agglutinins or complement-fixing antibodies. In no case have animals injected with sensitized vaccines shown the presence of protective antibodies. The protective antibodies we tested by injecting diminishing quantities of the rabbit serum with lethal doses of bacteria into mice.

Our conclusions from this experiment are that it is impossible to demonstrate the presence of antibodies in rabbits immunized with sensitized vaccines either living or dead. These results cannot be applied to immunization with all varieties of bacteria, because at present work in progress shows that agglutinins may be induced by the injection of sensitized pneumococci.

59 (1123)

## The effect of sodium citrate on blood coagulation in hemophilia.

By REUBEN OTTENBERG.

[*From the Pathological Department of Mt. Sinai Hospital.*]

The question of the effect on blood coagulation of the injection of sodium citrate into the circulation was raised immediately after the introduction of the citrate method of obtaining blood for transfusion.

Weil found that in cases with normal coagulation, the coagulation time immediately after citrate transfusions was slightly shortened instead of lengthened. As the question is one of particular importance in the hemorrhagic diseases and as there have been no observations recorded on the ultimate effect of citrate administration on the coagulation of blood, I wish to present some experiments in a case of hemophilia, whose prolonged coagulation time made it particularly suitable for this study.

The patient, an adult male, had nearly bled to death at least six times and presented all the typical features of the disease excepting the family history. The blood count showed nothing abnormal and the blood platelets were within the normal range or slightly above it (490,000 per cu. mm. counted in metaphosphate solution in a counting chamber). The coagulation time of his blood obtained at various intervals within the preceding three years had always been between one and two and a half hours.

The method of determining the coagulation time consisted in obtaining approximately three cubic centimeters of blood with a hollow needle direct from an arm vein. The blood was received into a clean five cubic centimeter test tube and observed at regular intervals, being kept at approximately body temperature. Complete coagulation was recorded when it was possible to turn the tube up-side down without the blood flowing. Beginging or partial coagulation was noted by the retarded flow of the blood when the tube was slanted. This method is far preferable to all the methods which involve the taking of drops of blood from the finger or ear as these methods, due to the admixture of fluids

from subcutaneous tissues, give notoriously inaccurate results. With the present patient on several occasions, blood so obtained and examined in capillary tubes coagulated in from ten to forty minutes at times when the venous blood was known to coagulate in one to two hours.

The injection of 150 cubic centimeters of normal blood from another person mixed with 0.3 gram of sodium citrate shortened the coagulation time of the patient's blood taken ten minutes after the transfusion from one hour fifteen minutes (beginning coagulation at fifty minutes) to seventeen minutes (beginning coagulation twelve minutes). Twenty-four hours later, however, the coagulation time was found to be practically the same as before the transfusion, namely one hour fifteen minutes for complete coagulation (beginning coagulation forty-five minutes). The coagulation time of blood obtained nine days later was one hour, thirty-five minutes (beginning coagulation one hour and twenty minutes).

The intravenous injection of 0.6 gram of sodium citrate (20 cubic centimeters of 3 per cent. citrate solution) shortened the coagulation time of the blood obtained ten minutes after the injection from one hour thirty-five minutes (one hour twenty minutes beginning coagulation) to twenty-five minutes. Forty-eight hours later, however, the coagulation time was found to have been lengthened out to two hours and fifty minutes (beginning coagulation one hour and twenty-five minutes).

At this time when the coagulation of the blood was at its longest, an experiment was made to see whether there was any immediate effect of citrate on coagulation after the citrate was injected into a muscle. The result was negative; the coagulation time taken thirty-five minutes after the intragluteal injection of 0.72 grams of sodium citrate was two hours and fifty-three minutes (beginning coagulation two hours). The coagulation time of the patient was not determined again for two weeks when it was found to have returned to approximately the same level as had been usual before the citrate injections, namely one hour (beginning coagulation forty-five minutes).

The citrate injections and the blood transfusion produced no ill effects whatever. The patient continued to have occasional

slight ecchymoses as before. Two months after the citrate injections he had another one of his attacks of severe hemorrhage.

### CONCLUSIONS

I. In hemophilia the intravenous injection of sodium citrate produces an immediate and great shortening of coagulation time which is followed, twenty-four to forty-eight hours later, by a return of coagulation time to its former prolonged period, or by a much greater prolongation of coagulation time than before.

II. The intramuscular injection of sodium citrate seems to have practically no immediate effect on coagulation time.

60 (1124)

## The influence of intravenous injections of magnesium sulphate upon the activities of the center of deglutition.

By J. AUER and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In order to understand our experimental results the following physiological facts have to be recalled. Three different phenomena which are under the reflex control of the center of deglutition must be distinguished: (1) The transmission of food from the mouth through the pharynx into the esophagus. This is a complex process which comprises the execution, in a coördinate and stable manner, of three separate activities: the closure of the entrances into the post-nasal cavity and into the larynx, and the rapid transportation of the contents of the mouth into the proper direction. We shall designate the entire action as the *initial act of deglutition*. The reflex mechanism which controls it, is more resistant to anesthesia than the two reflex mechanisms of the phenomena to be mentioned next. (2) The peristaltic movements of the esophagus. This is dependent only upon the occurrence of the first mentioned mechanism, the initial act of the deglutition, and is *independent* of the actual passing of some contents through the esophagus or of the anatomical continuity of the latter. Transection of the esophagus or complete removal of a

great part of it does not prevent the contraction in the lower part of the esophagus or of the cardia in due time after the initial deglutition, a time which varies with different species of animals (Mosso, Kronecker and Meltzer). The initial sensory impulse, after reaching the center of deglutition, passes consecutively through a number of sections of that center, sending, while thus passing, motor impulses to the corresponding sections of the esophagus. This reflex mechanism of *primary peristalsis* (Meltzer) is very resistant to fatigue, but is less resistant to anesthesia than any other of the reflexes with which we are here concerned. (3) Local reflexes of *secondary peristalsis* (Meltzer). A mechanical stimulus applied to any part of the mucosa of the esophagus (distension) will cause a contraction of the corresponding part of that canal, and, if this stimulus is brought about by some movable mass within the lumen of the esophagus, this mass will be driven down into the stomach by a wave which is difficult to distinguish from a wave of primary peristalsis. If the mass is mechanically prevented from being moved downward, no contraction takes place at any other part of the esophagus below the stimulating mass. That the secondary peristalsis is due to a central reflex and not to a peripheral mechanism, is proven by the fact that it disappears as soon as the vagi are cut. This central reflex is readily fatigued, but is, on the other hand, more resistant to central anesthesia than the transmission of the impulse from section to section within the center.

For several years we were engaged, at various times, in bringing forward evidences for the central nature of the inhibitory action of magnesium salts. With this object in mind, we studied in the present series of experiments the action of these salts upon the primary and the secondary peristalsis of the esophagus. The animals, dogs, received for anesthesia, three milligrams of morphin per kilo body weight. This permitted the operative procedures needed for our experiments, which consisted in exposing the trachea and making a window in it below the larynx; the transection of the esophagus and tying a glass tube in the upper end of it, the exposing of one superior laryngeal nerve and of tying a cannula in the external jugular vein. A short time after the operation the initial act of deglutition could be brought on by either of the three

following methods: by tickling the pharynx with a probe introduced through the window in the trachea, by injecting water or saline solution into the pharynx by the same route, or by electrical stimulation of the superior laryngeal nerve. The occurrence of peristaltic or local contractions of the various parts of the thoracic esophagus were observed by means of a catheter introduced into the stomach end of the esophagus. The catheter had around its esophageal end a small balloon of thin rubber; its outer end was connected with a water manometer. Magnesium sulphate was used in M/4 solution and was infused into the jugular vein from a Mariotte burette.

We shall report now our results very briefly. Before magnesium was given each initial act of deglutition was followed, as a rule, by a primary peristaltic contraction of every part of the thoracic esophagus. Further, stimulation of the esophagus, by moving of the catheter within the esophagus to a new place, or by a temporary distension of the balloon by air, brought on, as a rule, several consecutive contractions of the part in which the balloon was located (secondary peristalsis).

Some time after magnesium was permitted to run into the jugular vein we met first a phase in which the primary peristalsis disappeared, that is, no contraction of any part of the thoracic esophagus was observed to follow the initial act of deglutition. During this early stage the secondary peristalsis was in nearly all cases still present and quite normal; nor was the primary act of deglutition noticeably affected. When, however, more of the solution was infused, a stage was encountered in which also the secondary peristalsis was practically gone, while the initial act of deglutition was still only moderately weakened, and stimulation of the vagus still caused a fairly good contraction of the esophagus. A still further inflow of the magnesium solution finally greatly weakened, or even completely abolished, the initial act of deglutition also.

From these observations it is evident in the first place, that the first effect of the magnesium consists in a weakening or complete abolition of the primary peristalsis, which means that the inhibitory action of magnesium was exerted during this first phase exclusively or essentially upon the transmission of the sensory

impulse from section to section within the center of deglutition. The occurrence of efficient initial acts of deglutition and the presence of the secondary peristalsis testify that during this early stage the local reflexes within the center controlling the primary act of deglutition and the secondary peristalsis are little affected. This is in harmony with the fact that the mechanism in control of the two mentioned local reflexes are more resistant to anesthesia than the mechanism which controls the primary peristalsis. In the second phase also the local reflexes, controlling the secondary peristalsis, are abolished, while the initial act of deglutition is still fairly active. In this phase stimulation of the vagus causes a fairly good contraction of the esophagus. The facts observed during this phase permit the following two conclusions: (1) That the inhibitory action of magnesium in this phase is exerted essentially on the center and but little, if any, upon the motor nerve endings, and (2) that the local reflex of secondary peristalsis, which comes only occasionally into play, is more readily affected than the local reflex of the mechanism of the initial act of deglutition which is frequently in action and which has to be of a stable and resistant character. In the third phase, when the initial act of deglutition is also abolished, the inhibitory action of magnesium is probably exerted upon the center as well as upon the motor nerve endings. For our present purpose, however, it is of no interest to us to analyze the conditions prevailing during this phase.

The chief results of our experiments, so far as the action of magnesium is concerned, consists in the following conclusions: that a graded intravenous injection is capable of causing a complete central depression of the mechanism of deglutition before a peripheral effect can be ascertained; that the transmission of impulses from section to section *within* the center is more readily affected than reflex actions, and that reflexes of an important function in frequent action are more resistant than local reflexes of an incidental character.

61 (1125)

## Diabetes of maximum severity with marked improvement.

By **H. RAWLE GEYELIN** (by invitation).

[*From the Medical Clinic of the Presbyterian Hospital, Columbia University, New York.*]

Case on whom the following interesting observations were made is a man, nineteen years of age, with history of diabetes of six weeks' duration accompanied by extreme loss of weight (fifty pounds) and other classical symptoms of diabetes. Admitted to the hospital in condition bordering on coma.

Sugar output stationary for five fasting days. Symptoms slightly worse. Alternate fast and protein feedings accompanied disappearance of sugar in three weeks. During this period exhibited excessive nitrogen loss (from 25 to 38 gm. daily). Extreme acidosis and a dextrose: nitrogen ratio for three consecutive days of over 3.65.

Subsequently a tolerance of 250 gm. carbohydrate was obtained and four months after onset patient was tolerating 100 gm. carbohydrate on a mixed diet of protein, fat and carbohydrate, aggregating 2,500 to 3,000 calories daily. There was no acetone in the urine and the blood sugar remained normal (below 0.1 per cent.), the percentage when patient was first sugar-free having been .195 per cent., and on admission 0.312 per cent.

Just before discharge from hospital developed a peritonsillar abscess. Tolerance for carbohydrate markedly diminished in this period but rapidly returned after infection had subsided.

Points of unusual interest:

1. Most excessive continued nitrogen waste.
2. Highest D:N ever seen with recovery.
3. Acute onset of diabetes of great intensity, subsequent rapid development of high food tolerance with normal blood sugar.

62 (1126)

## The control of acidosis and its relation to impaired sugar metabolism in human diabetes.

By FRANK P. UNDERHILL.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.*]

Acidosis exerts a distinct influence upon carbohydrate metabolism. This assertion is supported by the observation of Elias,[1] who demonstrated that the introduction of acid into dogs and rabbits leads to hyperglycemia and glycosuria. Moreover, the same author[2] has concluded that the so-called "hunger diabetes" of young dogs[3] is in part, at least, a condition due to acidosis, as determined by the carbon dioxide content of the blood and analysis of the alveolar air. Observations upon human diabetes teach that acidosis obtains in this condition also.

A state of alkalosis is likewise potent in exerting an action upon carbohydrate metabolism but this influence is contrary to that of acidosis. Pavy and Godden[4] showed that the glycosuria provoked by ether and chloroform disappears after the intravenous injection of sodium carbonate. Given by mouth or intravenously sodium carbonate will abolish the hyperglycemia of "hunger diabetes" and glycosuria will either entirely disappear or be greatly diminished, according to Elias.[5] After removal of the pancreas sodium carbonate introduced into the blood stream causes diminution in the excretion of sugar.[6] Later work by Murlin (reported at the December meeting of the Society for Biological Chemists) has shown that under the influence of sodium carbonate the respiratory quotient is increased in depancreatized dogs. At the December meeting of the Society of Biological Chemists Underhill reported that in the hyperglycemia produced by epinephrine the

[1] *Biochem. Zeit.*, 1913, 48, p. 120.

[2] Elias, *Biochem. Zeit.*, 1913, 52, p. 331.

[3] Hofmeister, *Arch. f. Exper. Pathol. u. Pharm.*, 1890, 26, p. 355.

[4] *J. Physiol.*, 1911–12, 43, Proc., p. vii.

[5] *Biochem. Zeit.*, 1913, 52, p. 331.

[6] Murlin and Kramer, *J. Biol. Chem.*, 1913, 15, p. 365.

intravenous administration of sodium carbonate will significantly lower the excretion of sugar in the urine, the hyperglycemia being correspondingly decreased in height and duration. It was also stated that the intravenous injection of sodium carbonate into normal animals will sometimes although not invariably cause a distinct fall in the blood sugar content.

From these illustrative observations it may be concluded that a condition of acidosis tends toward the elimination of carbohydrate from the body whereas alkalosis shows a tendency to conserve the carbohydrate. Otherwise expressed it seems tenable that carbohydrate metabolism of the organism is maintained in equilibrium by a balance between the acids and bases of the body.

Applying these ideas to human diabetes one gains the following conception of its chemical pathology: without reference to what may initiate the abnormal condition, a state of acidosis unquestionably develops and must tend to become aggravated, if anything, by the characteristic acid-producing foods that characterize the conventional diabetic dietary. From what has already been pointed out, however, it seens reasonable to conclude that anything which will counteract or neutralize the continuous stream of acid entering the body should benefit the individual. One is led to ask, what influence would this have upon the excretion of sugar if the organism were once saturated, so to speak, with alkali and enough alkali continually supplied to neutralize the exogeneous and the endogenous acid? These considerations have been put to the test in a young diabetic, 26 years of age, with a very severe type of diabetes. When first seen by me fifteen months ago there was a sugar excretion of 151 grams per day. On a restricted diet the output of sugar was reduced to 25–50 grams, acetone and diacetic acid always being present in relatively large quantities. After a year's interval in spite of very stringent dietary restrictions the sugar excretion suddenly increased to 70–80 grams daily. Gradually increasing doses of sodium bicarbonate to a maximum of 120 grams per day resulted in a gradual diminution of sugar output until the urine became sugar-free. The dosage of sodium bicarbonate was thereupon decreased at the rate of 7 grams per day until the intake amounted to 42 grams which has been maintained to the present time. Under the alkali treatment the urine

has remained free from sugar for a period of seventeen days during which the food ingested has been augmented little by little to the point where about 10 grams of carbohydrate in addition to that present in the previous strict diet are being ingested daily. Throughout the entire course of his treatment the patient has continued at his duties as an instructor in the university.

63 (1127)

## Possible inter-relations between acidosis and creatine elimination.

By FRANK P. UNDERHILL.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven.*]

Current views associate the elimination of creatine with some perversion of carbohydrate metabolism. The probability of a close relationship of this sort is indicated by the well known fact that a deficiency of carbohydrate in the body leads to creatine elimination which may be checked promptly by ingestion of carbohydrate. There are experimental facts which the familiar hypothesis fails to explain. McCollum and Steenbock[1] found that in the pig a diet of corn products led to the appearance of relatively large quantities of creatine in the urine. Similar experiments of Folin (reported at the December meeting of the American Society of Biological Chemists) with oat feeding yielded comparable results. The dietaries employed can scarcely be regarded as lacking in carbohydrate.

Deficiency of carbohydrate usually means an accompanying acidosis, not necessarily caused by ketogenic substances, which presumably involve the tissues associated with creatine-creatinine metabolism. At any rate nearly every instance in which there is creatine in the urine is accompanied by an acidosis—generally a ketonuria also. These facts suggest the hypothesis that a condition of acidosis in the tissues is responsible for the appearance of creatine in the urine. To test it the following questions demand an answer.

[1] *J. Biol. Chem.*, 1912–13, **13**, p. 209.

1. Will creatine appear in the urine, even in the presence of an abundant carbohydrate supply, if acidosis is induced?

2. Will the elimination of creatine disappear if the acidosis is abolished, quite independently of the factor of carbohydrate supply?

Upon a diet of oats and corn, containing an adequate supply of carbohydrate, creatine promptly appears in the urine of the rabbit. A marked condition of acidosis, as measured by the hydrogen ion concentration of the urine, is always associated with this phenomenon. Oats and corn are pronounced acid-producing foods. On the other hand, if a base-producing food, such as carrots, is fed to rabbits with creatinuria this symptom rapidly disappears as the urine becomes strongly alkaline.

The protein *per se* is without special significance in the phenomenon under discussion; for upon a diet consisting of oats, corn and carrots creatine fails to appear in the urine, and the reaction of the latter remains alkaline. Equally significant is the further fact that the ingestion of HCl in addition to the mixed diet causes the appearance in the urine of significant quantities of creatine. Simultaneously the hydrogen ion concentration of the urine is markedly increased.

The conclusion seems inevitable that there is an inter-relationship between acidosis and creatine elimination. Creatine excretion may prove to be an index of a condition of acidosis in the organism.

64 (1128)

## On the production of soap jellies, and the physical conditions under which jelly formation takes place. (Preliminary communication.)

By G. H. A. CLOWES.

[*From the Biological-Chemical Laboratory of the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

In the course of experiments regarding the influence exerted by various electrolytes on the equilibrium of emulsions, published in the year 1913, the writer noted that NaCl, when used at a concentration in excess of .4M, caused a precipitation of some constituent

of the aqueous phase of an emulsion of oil dispersed in water, and that the emulsion subsequently broke down, the oil and water layers separating. This effect was believed to be attributable to the precipitation of the surface film of soap on which the stability of the emulsion depended. To test this question Na oleate was treated with salt at different concentrations, and it was found that at .4 to .45M NaCl complete precipitation of the soap took place. It was noted, however, that prior to precipitation a tendency to jelly formation was exhibited in the zone from .2M NaCl to .4 or .45M NaCl.

An attempt to repeat this experiment with a soap, which had been slightly acidified either by the addition of a minute quantity of oleic acid or of mineral acid, gave an entirely different result, an opalescence with increasing cloudiness and tendency to precipitation was noted between .2M and .4M NaCl, followed by complete precipitation at .45M NaCl. Further tests using varying proportions of soap, varying proportions of NaOH, and of NaCl and other salts of Na, brought out the remarkable fact that, as long as the soap employed was not too greatly diluted and was slightly alkaline, a jelly would be formed at all points between .2M Na and .45M Na regardless of whether the Na was derived from NaOH, from NaCl or other salts of Na.

In very concentrated soap solutions or in very strong alkali the jelly formation commences at a somewhat higher concentration and continues also somewhat above .45M. But it may be stated as a general principle that a zone of jelly formation obtains within these ranges, provided the original concentration of OH ions is in excess of the amount required to produce a strong pink coloration of the soap solution with phenolphthalein. If insufficient alkali is present as a result of the addition of small amounts of organic or mineral acids to the system, precipitation instead of jelly formation is observed. Since jelly formation commences and ends at a given strength of the Na salt almost regardless of the nature of the anions present provided there is a sufficient initial concentration of OH ions, it seems probable that the explanation is as follows:

A dispersion of Na oleate in water represents a dispersion of particles of oleic acid by means of NaOH. Further additions of NaOH lead to a more perfect dispersion of the soap particles,

owing to the fact that the OH ion is more readily adsorbed than the Na ion. NaCl exerts a similar effect to NaOH, the Cl ions exerting a dispersing effect analogous to that of the OH ions, but since they are far less readily adsorbed than the OH ions their effect is considerably smaller. This point may be demonstrated by adding NaCl in increasing amounts to a soap solution containing enough alkali to give a strong pink color with phenolphthalein. A discharge of the color takes place, and the amount of alkali required to compensate for the effect of the NaCl introduced follows a logarithmic curve indicating clearly that the added NaCl either promotes the adsorption of OH ions already present, or that the Cl ions are more readily adsorbed than the Na ions, thus leading to a reduction in the OH ion concentration in the water phase.

The soap particles possess a negative charge attributable presumably to adsorbed anions. This charge prevents their coalescence until the concentration of the Na ions reaches such a point that they also come into play and by adsorption on the particles tend to counteract or diminish the negative charge conveyed by the previously adsorbed OH or Cl ions.

When a certain concentration of the cation is reached, a critical zone commences, in which jelly formation or precipitation appears to depend entirely upon the relative proportions of adsorbed cations and anions. If at the commencement of this critical zone the residual negative charge carried by the particles resulting from an adsorption of anions in excess of cations is sufficient to maintain a perfect dispersion of the particles throughout the system, as indicated by an examination of the suspensions for Brownian movement by means of the ultramicroscope, jelly formation will ensue at higher concentrations. If this residual negative charge on the particles is insufficient, if they no longer exhibit perfect dispersion when examined by means of the ultramicroscope, if agglutination, aggregation and sedimentation under the influence of gravity has already commenced, precipitation necessarily ensues at higher concentrations. It is obvious, therefore, that the formation or non-formation of the jelly in this critical zone is dependent simply upon the relative concentration in the system at the lower critical point of anions like OH, which are more readily adsorbed and anions like Cl which are less readily

adsorbed, and more or less readily adsorbed cations. If at this critical point, the sum total of adsorbed anions is not sufficiently in excess of that of adsorbed cations to insure perfect dispersion, precipitation instead of jelly formation ensues. This explains the necessity for a certain minimum concentration of NaOH, with its readily adsorbed OH ions, to insure jelly formation in the case cited above.

It must be presumed that at the moment at which the particles suffer a sufficient loss of charge no longer to repell one another, they tend to coalesce with one another, and also become distorted and elongated into films and rods under the influence of changing surface tension conditions. It is obvious if they are sufficiently finely dispersed at this point that each particle will coalesce with its neighbor to form a jelly-like structure (analogous in a sense to a honey-comb) enclosing globules of water between the coalescing particles of the original dispersed phase, the structure retaining the form of the original containing vessel. If on the other hand the particles were not sufficiently dispersed at the time at which coalescences commenced, if they were already partly aggregated and no longer exhibiting perfect Brownian movement, they would no longer be perfectly distributed throughout the entire mixture, they would be further apart and, as a result of the diminution of their charge, would tend to aggregate and precipitate to the bottom of the vessel.

There are obviously a large variety of possible intermediary structures between the most perfect jelly formation, resembling a honeycomb, which would be impermeable to water, and the precipitated structure which would be absolutely permeable. Various degrees of permeability would result from the production of systems analogous to a sponge in which two continuous phases exist side by side, and the permeability of such systems would depend upon the extent to which intercommunication between adjacent partially enclosed aqueous phases has been maintained.

Further experiments with $CaCl_2$ and soap suspensions confirmed this theory and afford a satisfactory explanation for the phenomena of blood coagulation, the production of the casein clot, and other cases of jelly formation actuated by salts of Ca. The conversion of a system consisting of particles of fibrinogen dis-

persed in water, into a system consisting of a more or less perfect dispersion of water in an external or continuous fibrin phase may be further explained in a manner analogous to the explanation offered for the transformation of emulsions of oil in water into emulsions of water in oil, by considering the surface tension relations on both sides of a concentration film formed at the interface between the dispersed fibrinogen particles and the surrounding water. This phase of the question will be discussed in a subsequent paper on the process of blood coagulation.

This theory, that jelly formation depends on the extent of dispersion of colloidal aggregates when exposed to the effect of a precipitating agent, offers an explanation for the variations in permeability of a hypothetical protoplasmic membrane, or for that matter of tissues as a whole, under the influence of suboxidation products. A reduction in the concentration of OH ions available for adsorption resulting from the presence of acids would render the dispersion of certain colloidal aggregates less perfect than is normally the case. These aggregates would then tend to precipitate rather than to undergo jelly formation when subjected to the influence of coagulating agents. The structure formed would necessarily be more permeable and would possess less strength and elasticity than that formed under normal conditions of jelly formation. The destruction of emulsions and jellies, with resulting precipitation of the soap present when the concentration of NaCl exceeds .4M, probably bears some relation to the observation of Jacques Loeb that marine organisms are rapidly destroyed when exposed to that strength of NaCl, unless $CaCl_2$ or some other antagonistic salt is added.

The principle involved in the case of soap jellies considered above applies equally well to the reverse type of jelly formation where cations promote dispersion and anions exert an aggregating or precipitating effect.

The writer wishes to express his indebtedness to Miss Ruth Theis for her assistance in carrying out certain of the experiments referred to in this paper.

65 (1129)

## The hunger mechanism in birds. (Preliminary report.)

By F. T. ROGERS (by invitation).

[*From the Hull Physiological Laboratory of the University of Chicago.*]

Both normal and decerebrate pigeons have been used in this study. Hunger is marked by the appearance of restlessness. This restlessness appears before the crop is completely empty. Lack of water even though the crop be distended with dry food is marked by restlessness of the bird. These things are true of birds with cerebrum intact, partially, or wholly removed.

During hunger, changes occur in the behavior of the crop. In the normal bird with "appetite"(?) satisfied or at least in the bird which does not of its own accord eat of an abundant food supply, the crop is very much distended. In this condition only occasional contractions of the organ can be detected by means of a rubber balloon; none are visible to the eye (after removal of the feathers over the crop). An hour or two after feeding there begins to appear in the crop contractions in groups of three or four at intervals of 15–20 minutes. The activity of the crop is gradually augmented and 8–12 hours later there occur groups of 8–20 contractions at intervals of 10–30 minutes. Still later in some birds (probably young) the crop is in a state of almost continuous activity. When the content of the crop has been lessened to about one third of its capacity these contractions are directly visible. At this time they may be seen to involve principally the lower part of the crop. When it is completely empty these contractions are periodic in groups of 8–16 occurring at intervals of 10–60 minutes. Each contraction may be seen to begin at the upper part of the crop and sweep as a deep constriction, preceded by a marked bulging or relaxation, over the entire crop (and probably down to the gizzard). Each wave requires a time interval of 12 to 15 seconds to complete its cycle.

This visual evidence justifies the balloon method of recording the contractions. Unless the pressure used is excessive the balloon does not initiate the contractions.

In the crop which contains plenty of food and water a sudden distension of the balloon has little effect. Sudden distension of a balloon in an empty crop initiates a group of contractions. Using too big a balloon or using too much pressure so as to cause excessive dilatation of the crop causes sideways shifting of the neck and crop (shrugging of the shoulders so to speak) evidently an effort on the part of the animal to remove the obstruction. Similar movements may be seen in normal birds which have stuffed themselves with corn. By mechanical manipulation of the crop with the fingers isolated peristaltic contractions of the crop may be caused. Mere stretching movements of the neck are not sufficient to account for these contractions for they occur when the bird is held quietly in the hand.

In the normal bird these contractions may be inhibited by external influences such as light and noise. Light and sound do not inhibit them in decerebrate birds but rough handling may do so. Such disturbances of body coördination as those following extirpation of the semicircular canals or lesions of the cerebellum inhibit the contractions of the crop. Incidentally, during the period of marked incoördination following lesions of the semicircular canals or cerebellum the crop is emptied much more slowly than in normal birds.

Tonus changes undoubtedly occur but tracings are likely to be deceptive on this point because of the close relation of the crop to the cervical muscles. Any shifting in the position of the head will be registered by the recording balloon in the crop. Hence tracings may be meaningless. But in the hungry bird the crop can be seen to be constricted into a much smaller area. It can hardly be believed that the crop is simply folded and fallen together. (Histological study of the crop distended and empty is being made.)

A small fistula in the crop does not cause any visible difference in the contractions. The contractions may be inhibited by putting water into the crop through the fistula or by feeding the bird. Water given by mouth does not immediately inhibit the contractions for the peristaltic waves from the throat spread downward over the entire crop. No visible difference can be made out between contractions of the empty crop initiated by swallowing

water or those occurring perioically without swallowing, except as to their point of origin.

Restlessness of the starved decerebrate bird may be clearly periodic or more or less continuous. If it tends to be continuous picking up the bird and holding it in the hand for a moment and then freeing it will end the restlessness, unless contractions of the crop are occurring at the same time. If the crop is actively contracting the bird will continue his fruitless wanderings.

66 (1130)

## Oxygen consumption in regenerating tissue.

By G. G. SCOTT.

[*From the United States Fisheries Biological Station, Woods Hole, Mass.*[1]]

Little knowledge has been obtained as to the rate of metabolism of regenerating tissue as compared with that of normal tissue. Child, '15,[2] has found that susceptibility or physiological resistance of organisms varies directly with the rate of metabolism. He found, in practice, that a measure of the resistance to cyanide poison was an efficient method for determining the rate of metabolism. In experiments of regenerating tissue of *Planaria* (flatworm) he concluded that immediately after operation, the rate of metabolism fell below normal, remained there for a few days, then arose above normal where it remained for some time after regeneration was complete, when it gradually approached normal. I obtained the same result with *Sagartia*, a small *anemone* (Coelenterate). In my method the rate of metabolism was measured by determining the amount of oxygen consumed by the regenerating animals as compared with the normal animals. Oxygen determinations were made by means of the Winkler method. The experiment continued for twelve days. Determinations were made every twelve hours. Table I shows percentage consumption

[1] Published by permission of Commissioner of Fisheries.

[2] Child, C. M., "Senescence and Rejuvenescence," University of Chicago Press, 1915.

of oxygen by regenerating animals as compared with normal animals for each twelve hour period.

TABLE I.

1. Reg. *Sagartia* consumed 109% of amount of oxygen consumed by nor. *Sagartia.*
2. Reg. *Sagartia* consumed 96% of amount of oxygen consumed by nor. *Sagartia.*
3. Reg. *Sagartia* consumed 82% of amount of oxygen consumed by nor. *Sagartia.*
4. Reg. *Sagartia* consumed 95% of amount of oxygen consumed by nor. *Sagartia.*
5. Reg. *Sagartia* consumed 97% of amount of oxygen consumed by nor. *Sagartia.*
6. Reg. *Sagartia* consumed 93% of amount of oxygen consumed by nor. *Sagartia.*
7. Reg. *Sagartia* consumed 99% of amount of oxygen consumed by nor. *Sagartia.*
8. Reg. *Sagartia* consumed 111% of amount of oxygen consumed by nor. *Sagartia.*
9. Reg. *Sagartia* consumed 165% of amount of oxygen consumed by nor. *Sagartia.*
10. Reg. *Sagartia* consumed 142% of amount of oxygen consumed by nor. *Sagartia.*
11. Reg. *Sagartia* consumed 140% of amount of oxygen consumed by nor. *Sagartia.*
12. Reg. *Sagartia* consumed 135% of amount of oxygen consumed by nor. *Sagartia.*
13. Reg. *Sagartia* consumed 117% of amount of oxygen consumed by nor. *Sagartia.*
14. Reg. *Sagartia* consumed 130% of amount of oxygen consumed by nor. *Sagartia.*
15. Reg. *Sagartia* consumed 187% of amount of oxygen consumed by nor. *Sagartia.*
16. Reg. *Sagartia* consumed 135% of amount of oxygen consumed by nor. *Sagartia.*
17. Reg. *Sagartia* consumed 125% of amount of oxygen consumed by nor. *Sagartia.*
18. Reg. *Sagartia* consumed 124% of amount of oxygen consumed by nor. *Sagartia.*
19. Reg. *Sagartia* consumed 144% of amount of oxygen consumed by nor. *Sagartia.*
20. Reg. *Sagartia* consumed 129% of amount of oxygen consumed by nor. *Sagartia.*
21. Reg. *Sagartia* consumed 125% of amount of oxygen consumed by nor. *Sagartia.*
22. Reg. *Sagartia* consumed 119% of amount of oxygen consumed by nor. *Sagartia.*
23. Reg. *Sagartia* consumed 122% of amount of oxygen consumed by nor. *Sagartia.*

The result is parallel to that found by Child with *Planaria.* While extensive morphological studies on regeneration have been made, it is necessary that a more complete study of the physiological processes involved should also be made.

# SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

**Seventy-fifth meeting.**

*College of Physicians and Surgeons, April 19, 1916.*
*President Jacques Loeb in the chair.*

67 (1131).

## An active expiratory muscle in the chicken which is inhibited by stimulation of the central end of the vagus. A demonstration.

By **A. L. MEYER** and **S. J. MELTZER.**

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.*]

In mammals expiration is passive under ordinary conditions. It is only under abnormal conditions that certain muscles become active during the expiratory phase of respiration. At the last meeting of the Federation of American Societies for Experimental Biology[1] we made the statement that in the fowl normal expiration is active. We wish to demonstrate the truth of this statement by a graphic method. We have found that the innermost of the abdominal muscles in the chicken when carefully isolated contracts regularly with each expiration. When the contractions of this muscle are recorded simultaneously with the movements of the thorax it will be observed that the muscle contracts during expiration and suddenly relaxes during the onset of inspiration.

The literature concerning the effect upon the respiration of stimulation of the central end of the vagus in mammals is very extensive and full of conflicting opinion as to the nature of this effect. In fowls stimulation of the central end of the vagus causes an unmistakable inhibition of the contractions of this muscle. When the movements of the thorax and the contractions of the

[1] *American Jour. of Physiology* (Proceedings), 1916, 40 (No. 1), 127.

expiratory muscle are registered simultaneously, stimulation of the central end of the vagus brings out an instructive picture. Throughout the period of stimulation the thorax remains quiescent in an inspiratory position, while the expiratory muscle remains completely relaxed.

This phenomenon is another instance of the general law of "contrary innervation" (Meltzer), or "reciprocal innervation" (Sherrington). Inhibition of the expiratory group of muscles during inspiration was suggested by one of us over thirty years ago.[1]

68 (1132)

## A demonstration of the effects of some lesions of the nervous system.

By J. GORDON WILSON and F. H. PIKE.

[*From the Department of Otology, Northwestern University, and the Department of Physiology, Columbia University.*]

The effects of the lesions were shown in cinematograph films of three different animals. A rabbit which was brought into the laboratory some months ago presented constant marked torsion of the head to the *left*. There was no nystagmus, but merely a constant deviation of the eyes. The animal could move about on rough surfaces if it went slowly and carefully, or if its left side was supported by the side of the cage. If put on a smooth surface with the left side unsupported, any attempt on the part of the animal to move was followed by rolling movements to the left, about the long axis of the body. If no obstacle was placed in its way, the animal might roll for several yards before regaining its upright position. The animal was said to be about eight months old at the time it was brought into the laboratory, and to have been in the same condition from birth. The only gross changes visible at autopsy were in the left otic labyrinth. The nature of these changes was not determined by inspection. The histological report will be presented later. One interesting point in the deportment of the rabbit was its lack of compensation for the loss

[1] *Arch. für Physiol.* (DuBois-Reymond's) 1883, 216.

of the labyrinth, as compared with the deportment of cats or dogs after loss of one labyrinth.

Two cats were subjected to experimental ablation of the vermis and left lateral lobe of the cerebellum. The eye movements were different from those following labyrinthine lesions. One marked motor defect was the trembling and uncertainty of movement of the head when attempting to take food. Two different stages in recovery from the effects of the cerebellar lesion were shown, in one of the cats, with the gradual amelioration of the symptoms in the second stage taken at an interval of about one month after the first.

The film of the rabbit was made through the courtesy of Pathe Freres. The films of the cats were paid for out of the Patton Fund of Northwestern University Medical School.

69 (1133).

## A separation of serum into coagulative and non-coagulative fractions.

By ALFRED F. HESS.

[*From the Research Laboratory, Department of Health, New York City.*]

As is well known, it is possible, by means of salting out with appropriate percentages of ammonium sulphate or sodium chloride, to almost entirely separate the albumin from the globulin and the pseudo-globulin of serum. This has been done in the preparation of diphtheria antitoxin, where it has been found that the antitoxin is closely associated with the pseudo-globulin fraction.

A similar procedure was carried out to determine the association of the coagulative principles of the serum. It was found that in human plasma as well as in horse plasma, these substances are linked with the euglobulin fraction. If these three proteid fractions of the serum are separated and dissolved in normal salt solution and added to plasma (with the addition of a small amount of calcium) the euglobulin will markedly hasten coagulation, whereas the two other fractions will have either no effect or a slightly inhibitory action. It is possible in this way to prepare a

refined serum containing only ½ to 1 per cent. proteid, that is to say, one tenth to one fifth the quantity present in normal serum, but possessing an equal potency as regards coagulation. This preparation may be passed through a Berkefeld filter so as to be rendered sterile.

This euglobulin would seem to be of value for subcutaneous or intravenous use in hemorrhage, particularly on account of its small quantity of proteid.

70 (1134)

## Comparative distribution of urea, creatinine, creatine, uric acid, and sugar in blood and spinal fluid.

By **M. S. FINE** and **V. C. MYERS.**

[*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital.*]

Comparative analyses of blood and spinal fluid were carried out in 15 cases. These patients were suffering from nephritis of various stages of severity, and gave chemical blood pictures varying from practically normal to the excessive retention of uremia. The concentration of urea in the spinal fluid averaged 88 per cent. of that in the blood; the concentration of creatinine, 46 per cent.; of creatine, 22 per cent.; and of uric acid, 5 per cent. of the respective concentrations in the blood. If these differences in concentrations may be regarded as representing the relative permeability of the cells separating the blood and spinal fluid, one notes that the extent of passage into the spinal fluid is greatest for urea, less for creatinine, still less for creatine and least for uric acid. It is of interest to note that this represents also the order of their solubility in water, and, in part, the relative ease with which these substances appear to be eliminated by the kidney.

It may be further observed that the sugar concentrations of the spinal fluid in these fifteen cases averaged 57 per cent. as much as that of the blood.

71 (1135)

## Antagonism between atropin and certain central emetics.

By CARY EGGLESTON.

[*Laboratory of Pharmacology, Cornell Medical College, New York City.*]

The minimal certainly emetic vein dose of pilocarpin alkaloid (hydrochloride used) was determined for dogs as 0.7 mg. per kilo. It having previously been observed that atropin was capable of antagonizing the emetic action of pilocarpin, experiments were made to determine the smallest dose of this alkaloid (sulphate used) which was just sufficient to prevent emesis from the minimal emetic dose of pilocarpin. The antagonistic dose of atropin alkaloid was then determined for twice, four, eight and sixteen times the minimal dose of pilocarpin.

The results showed that it requires about 1/75th as much atropin base as of pilocarpin base to antagonize the emetic action of the smallest effective dose of the latter. About the same ratio was found for twice the dose of pilocarpin. For four times the minimal dose of pilocarpin 1/95th as much atropin was required; for eight times about 1/125th; and for sixteen times about 1/200th.

Similar experiments were made with nicotin and atropin, but the toxicity of the former drug prevented the use of amounts larger than the minimal emetic dose. Atropin was found to antagonize nicotin in the proportion of about 1 : 70 (both in terms of base).

Other emetics previously shown to cause vomiting through central action were tested with atropin in doses up to 5.0 mg. of the base per kilo, or 500 times the effective dose against pilocarpin and 1,000 times that against nicotin. In no case was there any antagonism demonstrable. The drugs used were apomorphin, morphin, ouabain and emetin.

It has been shown[1] that pilocarpin produces emesis through a direct central action and since section of the vagi does not increase the minimal emetic dose, a local action of the drug in producing emesis seems very improbable. The antagonism of atropin,

[1] Eggleston, C. and Hatcher, R. A., *Jour. Pharm. and Exp. Ther.*, 1915, VII, 225.

therefore, would seem to be a central one, probably in the nature of a depression of certain central structures concerned with the vomiting act, or of certain paths to or from the central mechanism. It should be stated that the dose of atropin required to antagonize the minimal emetic dose of pilocarpin is insufficient to dilate the pupil and does not appreciably diminish the salivation or diarrhea produced by the pilocarpin. The mechanism of antagonism between atropin and nicotin is apparently the same as between atropin and pilocarpin, and it is interesting to recall the fact that nicotin and pilocarpin—the only central emetics which we have found so far to be antagonized by atropin—are very closely related in their pharmacologic actions.

Atropin is stated to be capable of preventing the emesis often seen following the therapeutic use of morphin in man and that induced in dogs. The mechanism of this action is usually given as involving a local action of both drugs on the stomach, morphin emesis being ascribed largely to a marked stimulation of the motor endings of the vagus in the stomach, which are depressed by atropin. In dogs, at least, morphin has been shown[1] to produce emesis through a central action and we have not been able to prevent this action by atropin in any dose. This failure confirms the observations of Guinard,[2] who, however, conceded some antagonistic action between atropin and morphin in man, which he thought due to a synergistic central depressant action of the two drugs.

The failure of atropin to antagonize the central emetics studied, other than pilocarpin and nicotin, raises several interesting points regarding the physiology of vomiting. We are all aware of the number and diversity of ways by which vomiting may be induced and of the existence, therefore, of many afferent paths for the stimulation of the central vomiting mechanism. It is suggested, on the basis of the present observations, that atropin antagonizes nicotin and pilocarpin on the one hand by depressing some limited portion of the vomiting center, and on the other hand fails to antagonize the other centrally acting emetics used since these may possibly influence the central mechanism through other and different portions.

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[1] *Loc. cit.*

[2] *Lyon Medical*, 1895, LXXX, pp. 37 and 49.

Experiments were also conducted using hyoscyamin in place of atropin, and others are now under way covering some of the other drugs with central emetic actions. The results of all of these will be detailed in the complete paper to be published later.

72 (1136)

## The distribution of the fat soluble A, the growth-promoting substance of butter fat, in the naturally occurring foodstuffs.[1]

By E. V. McCOLLUM, NINA SIMMONDS, and WALTER PITZ (by invitation).

[*From the Laboratory of Agricultural Chemistry of the Wisconsin Experiment Station.*]

That butter fat and egg yolk fats contain a substance whose chemical nature is unknown, which is indispensable for growth or prolonged maintenance of health was first pointed out by McCollum and Davis. Later they showed the presence of this substance in the maize kernel and in wheat embryo, and presented some evidence that if it is found in the oat kernel it is in very small amount.[2] Our further studies have confirmed these observations.

McCollum and Kennedy[3] have discussed the desirability of employing the term "fat-soluble A" for this, to distinguish it from the "water-soluble B," a substance which is widely distributed in the natural foodstuffs of both animal and vegetable origin and is likewise indispensable for growth or prolonged maintenance. The water-soluble B only is concerned with the production and cure of polyneuritis in pigeons.

Our experimental work with the grains has shown that the content of the fat-soluble A is greater in the maize kernel than in wheat, and greater in wheat than in the oat kernel. In all three the content is too low to induce growth at the maximum rate even though all other factors in the diet be near the optimum.

[1] Published with the permission of the Director of the Wisconsin Experiment Station.

[2] McCollum and Davis, *Jour. Biol. Chem.*, Vol. 15, p. 167 (1913); Vol. 21, p. 179 (1915); Vol. 23, pp. 181 and 231 (1915).

[3] McCollum and Kennedy, ibid., vol. 24, p. 491 (1916).

We have much experimental evidence indicating that the unknown A is principally confined to the germ of the seed. Sunflower seed appears to be fairly rich in this substance.

We have also found that the leaves of certain plants, especially alfalfa and cabbage are very rich in the fat-soluble A as compared with the grains. It is probable therefore that it is universally associated with metabolizing plant cells. We have rats in our colony which have grown to very near the normal adult size at slightly below the normal rate on a simple mixture of polished rice sixty and powdered alfalfa leaves forty per cent. They are in an excellent condition after eight months on this diet and one female has produced young.

We wish to call attention to the importance of having found a good source of the fat-soluble A in foodstuffs containing but little fats and other substances soluble in lipoid solvents. We shall report later on methods of isolating this substance from such sources.

73 (1137)

**The effect of exercise on the blood sugar of depancreatized dogs.**

By GEORGE M. MACKENZIE (by invitation.)

[*From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.*]

Blood sugar curves of dogs made to run on the treadmill one to four days after extirpation of the pancreas showed:

1. That after 20 to 30 minutes of such exercise, in animals which were being fed 200 grams of meat and bread daily, there occurred a fall in the amount of reducing substance in the blood, sometimes amounting to as much as 100 mgm. per 100 c.c.

2. That in starved animals such exercise caused a rise in the amount of reducing substance in the blood, amounting in one case to 85 mgm. per 100 c.c. during 30 minutes of exercise.

The conclusions suggested by these results are that, even after complete extirpation of the pancreas the power of sugar consumption is not entirely lost, and that there may be a difference in the power of such animals to utilize sugar according as it is derived from tissue proteins or by absorption from the intestinal tract.

74 (1138)

## Studies on the blood of the albino rat. Its normal cellular constituents. Their reaction to sarcoma growth and to benzol treatment.

By KENNETH TAYLOR, M.A., M.D. (by invitation.)

[*From the Laboratories of the Department of Medicine, University of Minnesota.*]

While working with a transmissible sarcoma of the white rat it was found desirable to note the changes in the blood picture during the growth of the tumor. With the idea of establishing the normal, a careful search of the available literature on the blood of the albino rat (and also the common wild rat) was made. No reports on the blood plates and only a few on the leucocytes of this animal could be found. For this reason, and because of the growing importance of the white rat as a laboratory animal and its availability for tumor work, it has seemed advisable to contribute this small series of studies on the blood of albino rats, with special reference to the blood plates in the normal animal. The changes in the blood picture due to sarcoma growth and benzol treatment have been observed.

Hans Hirschfeld[1] in a paper on the differential morphology of the white cells of the blood reported the usual types of cells to be present in the blood of rats of mixed and albino breeds. He noted especially a cell with annular nucleus and fine eosinophilic granules. He did not report the usual number of white cells or their differential count.

Pappenheim[2] was the first to report in brief the changes in the blood of the albino rat coincident with the progress of fatal transmissible sarcoma. His conclusions were that, except for a very slight secondary anemia and a great degree of polychromatophilia and granular degeneration of the red cells, there was little reaction on the part of the blood until the tumor ulcerated, when a marked leucocytosis appeared.

[1] *Virchow Archiv*, 1897, CXLIX, p. 22.

[2] *Folia Hematol.*, Vol. X, 2, p. 393.

Hirschfeld[1] reached much the same conclusions, but reported finding normoblasts in the blood. He recorded a leucocyte count of 180,000 in one rat with an ulcerating sarcoma. No normal standard of number or of differential count of leucocytes was reported in either of these papers. The blood plates were not enumerated or described.

In the work presented here the leucocytes and blood plates were counted after the method of Wright and Kinnicutt[2] designed for plate counting. It was modified by the procedure of first drawing the fixing fluid to the mark .5 in the stem of the white cell pipette in order to prevent the blood from coming in contact with a dry surface. A 1–20 dilution was invariably used. Two pipettes were used for each count and five drops counted from each to determine the number of leucocytes. One hundred small squares (Turk's stage) from the dilutions in each pipette were counted to determine the number of blood plates. The following table shows the results of eighteen studies on the blood of eight apparently healthy albino rats. For the blood plates the figures given represent the nearest 50,000; for the leucocytes the nearest 500.

Average number blood plates per ccm..... 1,000,000
(Variation 850,000 to 1,200,000)
Average number of leucocytes per ccm..... 19,000
(Variation 12,000 to 30,000)
Average number of red blood cells per ccm.....10,000,000
(Variation 9,000,000 to 10,500,000)

Differential leucocytes (300 cells counted):

Average polymorphonuclear cells.....50 per cent.
(Variation 30 per cent.–60 per cent.)
Average mononuclear cells (small 30 per cent., large 14 per cent.) . 44 per cent.
(Variation in proportion great)
Average transitional cells..... 5 per cent.
(Variation 2 per cent.–14 per cent.)
Average eosinophil cells..... 1 per cent.
(Variation 0–3 per cent.)

It will be seen from the table for normal rats that the blood plate count shows an unusually small degree of variations. The

[1] *Fol. Hemat.*, Vol. X, 2, p. 393.
[2] *Jr. A.M.A.*, 1911, LVI, p. 1457.

plates themselves, however, differ greatly in size, measuring from two to five micra in long diameter. In the diluting fluid they usually appear slightly oval and cup-shaped. They are granular. Varied forms, however, may be seen in the dry and stained preparations. The red blood cells, even in healthy rats, always show considerable granular degeneration and polychromatophilia. The total number of leucocytes is high as compared with the human count and, as shown in the table, has a wide variation in apparently normal animals. The reaction to an inflammatory process commonly results in a high leucocytosis. Several counts of over 45,000 per c.c. were made. Two were in cases of large subcutaneous abscesses, two in cases of ulcerating sarcoma.

The differential count of leucocytes in normal animals shows a fairly constant percentage of polymorphonuclear cells. The total number of mononuclear cells is also fairly definite, but there is great variation in the proportion of large and small cells in different animals. While the small mononuclear cells usually greatly outnumber the large, two apparently normal rats showed more large than small cells.

In five albino rats inoculated with sarcoma the blood was observed. The results agree in general with those of Pappenheim and of Hirschfeld. No marked change in the blood picture is noticeable during the growth of the tumor except an increase in the amount of polychromatophilia of the red cells and a slight increase of small mononuclear leucocytes at the expense of the large mononuclear and transitional cells. When the tumor was of an ulcerating character, however, leucocytosis was marked: 45,000 in one, 50,000 in another rat. Blood plates remained stationary.

Benzol leucopemia was readily produced in two rats, the leucocyte count falling from 15,000 to 1,200 in one, and from 25,000 to 2,200 in the other; while the plates showed a corresponding drop from 850,000 to 500,000, and from 1,000,000 to 500,000 respectively inside of ten days. The fall in leucocytes was proportionately greater in the large mononuclear and transitional cells than in the polymorphonuclear and small mononuclear cells.

SUMMARY.

1. In the blood of the normal albino rat the blood plate count approximates 1,000,000 per c.c. and is fairly constant.

2. The total leucocyte count is variable and reacts violently to ulcerative processes.

3. The reaction of the cellular elements of the blood to the growth of transmissible sarcoma is slight and probably in no way characteristic.

4. Benzol injections produce a more rapid and proportionately greater reduction in the leucocytes of the blood than in the plates.

75 (1139)

## Gravimetric determination of beta-oxybutyric acid.

By DONALD D. VAN SLYKE.

[*From the Department of Chemistry of the Rockefeller Institute.*]

If beta-oxybutyric acid is oxidized with dichromate in the presence of sulfuric acid and mercuric sulfate, a precipitate of the acetone compound of mercury sulfate can be obtained in an amount proportional to the beta-oxybutyric present. Thus, if 175 c.c. of a beta-oxybutyric solution containing 9 per cent. of sulfuric acid, 2 per cent. of mercuric sulfate, and 0.25 gram of potassium dichromate are boiled under a reflex for an hour, 7.7 milligrams of mercury-acetone compound are precipitated for each milligram of beta-oxybutyric acid present. The beta-oxybutyric acid may vary from 1 to 9 mg. without affecting the ratio, if the concentrations of the other reagents are kept constant.

76 (1140)

## Complement fixation in tuberculosis.

By H. R. MILLER, M.D. and HANS ZINSSER, M.D.

[*From the Department of Bacteriology, College of P. and S., Columbia University, New York.*]

In a recent communication to the New York Pathological Society, the material of which is to appear in the *American Journal*

*of Medical Sciences*, the writers described work on complement-fixation in tuberculosis, carried out with a very simple antigen which had yielded and is still yielding results more satisfactory than those hitherto reported by other workers who had used other antigens. The work followed a study of culture-filtrate antigens, such as those devised by Besredka and by Petroff, and the special modifications of the Besredka medium employed by Bronfen-Brenner and by Craig. These antigens did not in our hands react with the regularity which we thought should attend a reaction of specific diagnostic value. Owing to irregularities perhaps due to constituents of the media, it was thought wise to return to the bacillary substances themselves, work along this line having been attended by considerable success within recent years—notably in the hands of Radcliffe, Dudgeon, Weir, and Stimson. It should not be forgotten that the same direction of investigation was followed in the earlier work of Wassermann and Citron and in that of Calmette.

The method employed is in general identical with that which we have been using in this laboratory for the extraction of *Treponema pallidum*, typhoid bacilli and streptococci, and differs in no essential particular from the so-called "endotoxin" extraction method employed by Besredka in 1906 with organisms of the typhoid-colon group. Since we feel that the procedure at present in use in the Columbia laboratory should be thoroughly reinvestigated by other workers, we believe that it is proper to give in great detail the method by which the antigen is made.

The bacilli which, so far, have been used for the production of the antigen have been of the human type, some of them isolated by Miller, some of them obtained from Professor Theobald Smith, some from the laboratory of Professor William H. Park, and some from the laboratory of Parke Davis & Co. They have been grown mainly on the gentian-violet medium of Petroff and on Miller's modification of this medium; also on Petroff's potato broth. It is at present the impression of the writers that the medium on which the bacilli are grown plays no great part in determining the usefulness of the antigen. It seems, however, to be important that a number of different strains should be used—that is, that the antigen should be polyvalent—and the use of relatively young

cultures is advisable. So far, in most of the reactions, unheated bacteria have been used. Inasmuch as the method of production, under these circumstances, is fraught with a not inconsiderable element of danger, we have recently begun to use bacteria heated to 60° for a half hour, and, in the one series so carried out, no deteriorating effect of the heating was apparent. These problems of detail, as well as many others, are being more thoroughly investigated.

20 mgm. of the moist tubercle-bacillus mass are weighed out, placed in a conical 15 c.c. centrifuge tube, and to it are added 90 mgm. of table salt. With a glass rod, filed to roughness at the end, this paste is ground by hand for about one hour. Distilled water is then added to isotonicity; that is, 10 c.c. to the quantities above described. This is the antigen. Just before using, it is shaken up and the heavier particles are allowed to settle in the course of a few minutes. Except for the removal of these larger elements, the suspension is used as a whole without centrifugation and without filtration.

The antigen so prepared has hardly ever been found anticomplementary in quantities as large as 1 c.c. and has given fixation with positive sera (the inactivated sera used in quantities of 0.1 c.c.) in amounts as low as 0.02 c.c. The titrations, as well as the reactions, have been done with one half the original Wassermann quantities, using a sensitization of two units of amboceptor and two units of complement. So far, we have used the anti-sheep rabbit hemolytic system. As a routine, the 37° one-hour water-bath incubation has been employed. A number of parallel series have been done by the four-hour ice-box method, but, since this seems to make little difference in the results, the time-saving 37° method was decided upon as a routine procedure. The antigen appears to be quite stable. We have used with satisfaction antigens as old as six or seven weeks, kept on ice.

We are ready to report the results of 602 cases tested. Of these 103 were negative for tuberculosis; that is to say, they represent patients in whom, clinically, tuberculosis was excluded. 226 cases were clinically diagnosed as actively tubercular. Their sera tested gave the following results:

"Stage one" cases: 32 in all with active clinical symptoms gave

positive fixations. Tubercle bacilli were not found in the sputum from 16 of these cases. 7 of these 16 were suffering from the early incipient type of the disease.

"Stage two": We tested the sera of 110 such cases. All but 12 in this series had positive bacteriological proof of infection. The fixation test was positive in all but 2 cases.

"Stage three": 84 cases tested. One very advanced case gave no fixation. The sputum was positive in all but one case. The fixation test was positive in 83 of these 84 third-stage cases.

We have, then, 226 patients suffering from clinically active tuberculosis, in whom the test was positive in 223 cases.

The reaction was done with 88 sera from so-called *healed* (arrested or inactive) cases. These were cases which, at one time or another, had suffered from active tuberculosis but which were, at present, apparently free from symptoms pointing to absorption from any active focus. 54 cases were negative for the test; 13 cases were positive, however in 8 of these tubercle bacilli were found in the sputum shortly before the test was performed; 21 cases showed weak fixation. Here we have a group of 88 healed, or better, arrested cases in which the reaction was negative in 54, weak in 21, and positive in 13, 8 of these 13 being cases with positive sputa.

Our next group consists of 140 doubtful cases where no diagnosis was established and where, obviously, no bacteriological proof was present. 32 in this group gave positive fixation; 108 negative. In some of the 32 positive cases, a definite clinical diagnosis of tuberculosis was subsequently established. In none of the 108 negative cases has there been found, thus far, any evidence of tuberculosis. This group of doubtful cases includes 84 pulmonary cases, 5 glandular cases (3 of which were diagnosed as Hodgkin's disease. In one of the Hodgkin cases, the serum report was ++++, and an excised lymph node from this patient turned out to be tubercular upon later pathological examination); also there were 21 eye cases, 1 case of sepsis of the throat, and 12 miscellaneous cases.

45 positive Wassermann sera were tested. 2 gave positive fixation with the tubercle bacillus antigen. One of these two cases was a dispensary patient who at present can not be followed

up; the other was a patient with tuberculous peritonitis who had had lues.

Almost all the healed cases had positive skin tests, yet 54 of the total series of 88 showed negative fixation; 24 of the 103 cases in which tuberculosis had been excluded gave marked intradermic reaction to tuberculin. The fixation test was negative in these 24 also.

In the foregoing communication, we have reported an antigen for complement-fixation in tuberculosis which has seemed to give us results more regular and satisfactory than those reported by other workers and which has the advantages of great simplicity. The nature of the reaction and its results incline us to believe that we are dealing with a specific reaction which depends upon the presence or absence of antibodies to the tubercle bacillus in the circulation of the patient. It is well to bear this in mind in judging the results of the reaction, since it must not be forgotten that specific complement fixation may not be a direct measure of infection, but rather indirectly it may point to the invasion of the body by a specific microörganism, by determining the presence of antibodies. Thus, it may be too much to expect, especially in a disease so chronic as tuberculosis, to find antibodies circulating in all forms and in all stages of the disease. Perhaps this will make it more easy to understand why the reaction has given positive results in active cases only, nearly always negative ones in inactive tuberculosis, and was occasionally negative when tubercle bacilli were in the sputum but the clinical condition was one of arrested disease. It is this aspect of the reaction particularly which leads us to hope that it will be of clinical value in indicating, not so much the existence of infection as of determining the activity of the focus, and, incidentally, giving us a method of studying the fluctuations of antibodies during the disease. The work will, of course, have to be continued by multiplying the number of cases already observed. We are also proceeding in our own laboratory to study the specificity of antigens made with bovine cultures and to study the relationship of this reaction to the diagnostic tuberculin tests.

77 (1141)

## Preliminary studies on the antigenic properties of different strains of bacillus typhosus.

By SANFORD B. HOOKER. (by invitation.)

[*From the Hearst Laboratory of Pathology and Bacteriology, University of California.*]

A search through the literature reveals no report of special work upon antigenic differences among typhoid strains, although serologic methods have frequently been used in differentiating typhoid from closely allied organisms. The demonstration of the severally specific antigenic individualities, notably of strains of pneumococci, streptococci, gonococci, meningococci, and influenza bacilli; the fluctuant epidemiologic severity of typhoid fever from time to time; the observation that antityphoid inoculation confers no protection against paratyphoid infection; the growing list of instances in which antityphoid inoculation has been unsuccessful, the previously known and personally confirmed fact that a polyvalent antigen is essential for good alexin fixation reactions in typhoid fever are the main facts which have led to this investigation.

Attention has been focused chiefly upon the delicately specific method of alexin fixation as a means of detecting antigenic differences. A considerable number of confirmatory agglutinin absorption experiments have also been performed.

*Materials and Technic.*—Of the 48 strains which have been used 21 are laboratory strains two to fifteen years old, and the rest have been isolated and authenticated during the past year by Gay and Chickering in the course of studies of local cases of typhoid fever.

The antigens used were washed, formalized suspensions of typhoid bacilli. These suspensions have been used also for immunizing rabbits, being eminently satisfactory for this purpose as agglutinogens for agglutination and absorption tests. The total volume of the fixation test has been one cubic centimeter, one fifth that of the classical Wassermann. Sheep cells, rabbit anti-sheep hemolysin, and guinea-pig serum make up the hemolytic system

which has daily been balanced by simultaneous alexin and hemolysin titrations. Pooled serum from six or more guinea pigs has been preserved by salting for alexin, a method which has many advantages.

Serum cross-titrations with standardized antigens have developed the fact that different strains of typhoid bacilli fall into different groups, somewhat analogous to the groupings of pneumococci. This is evidence which must be seriously considered in the explanation of the causes of failure in typhoid vaccination, since it is not unlikely that subjects may have been infected with strains dissimilar to those used for prophylactic inoculation. The strains tentatively placed in Group I cross-fix with all antigens; those in Group II cross-fix with each other but not with Group I strains. Group I-A strains give irregular results. There is no apparent relation between the virulence of the organisms as indicated by the severity of the disease which they caused, or between their toxicity as indicated by the reactions produced in immunizing rabbits, and this grouping. All of the Group II strains, however, have been under artificial cultivation for a number of years. Number 13 is the Rawlins strain, so extensively used in prophylactic immunization. It seems to possess a lesser antigenic complexity than does any organism in the other groups. On this evidence it would seem to be theoretically of less value as an immunizing strain.

The results of absorption tests, while somewhat less consistent, are confirmatory of the findings obtained with the alexin fixation reaction. Group I and Group II serum absorbed respectively with Group I and Group II strains give usually negative results with all organisms. Absorption of Group I sera with Group II strains results in the removal of all agglutinins for Group II while agglutinins for Group I still remain.

It is considered that the evidence of antigenic differences thus far discovered among typhoid strains is sufficiently valid to warrant the presentation of these data, and sufficiently encouraging to justify similar more extensive work, especially with regard to the comparative protection which is aroused by strains of different character. Although the principle of polyvalency has been used empirically in the past, it would seem advisable now on more

certain data to employ a polyvalent typhoid vaccine for immunizing and therapeutic purposes compounded in accordance with these groups as tentatively suggested and which may be confirmed or extended in the future.

78 (1142)

## Note on "Salt fever."

By THEO. C. BURNETT and GEO. H. MARTIN, JR.

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

About six years ago one of us[1] published a short account of the rise of temperature which follows the injection of sodium chloride into rabbits, either intravenously or subcutaneously. This fact seems to have been completely overlooked by subsequent investigators, of whom there are many (Bingel, Freund, Samelson, Hort and Penfold, McIntosh, Fildes and Dearborn, and others). Samelson[2] claims that the rise of temperature is due to bacterial toxins contained in the distilled water, and not to the sodium chloride. The observations were made on nursing children. Freund,[3] on the other hand, maintains that the sodium chloride is the cause of the rise of temperature, at the same time admitting the fact that contaminated water may also cause fever.

As we wished to make use of this fact in another connection, it became necessary for us to be sure that the rise of temperature was due to the injection of the salt, and to that end we have repeated the earlier work. Sterile sodium chloride was put in a flask that had been throughly sterilized, and the water, redistilled in glass, was received directly into the flask from the condenser. The mouth of the flask was closed with sterile cotton, and the solution (*m*/6 concentration) was used as soon as it had cooled down to the proper temperature. There can be no doubt, therefore, of the purity of the solution as far as bacterial contamination is concerned. Antiseptic precautions were observed in making the injections.

[1] Burnett, Univ. Calif. Publ., Vol. 4, 1910, p. 5.

[2] Samelson, *Monatsch. f. Kinderheilk*, Vol. 11, p. 3.

[3] Freund, *Arch. f. exp. Path. u. Pharm.*, Vol. 74, 1913, p. 311.

The results confirmed our earlier work. The temperature rose steadily after injection, the height being roughly proportional to the dose given. The maximum was attained in from three to five hours, after which the temperature gradually returned to normal. As an example, a rabbit weighing 2,500 grams was injected subcutaneously with 25 c.c. sterile sodium chloride solution, *m*/6. In five hours a maximum temperature of 40.4° C. was recorded. The next day 35 c.c. were injected, with a maximum of 41° C. in five hours. The following day 20 c.c. gave a maximum of 40° C. in three hours. 25 c.c. Ringer's solution caused a slight rise, but not so marked as the pure sodium chloride (39.8° as against 40.4°). This is not in accord with our original findings, and it is possible that the distilled water used at that time was not perfectly pure. Our present results are more in harmony with Loeb's theory of balanced solutions, and with the results of other workers.

As intravenous injections become more and more general, it would seem wise, when sodium chloride is used as a menstruum for other substances, that the amount injected should be so graduated as to fall below that which will cause a febrile reaction; or better still, Ringer's solution should be used.

79 (1143)

## The influence of morphin upon the elimination of intravenously injected dextrose.

By I. S. KLEINER and S. J. MELTZER.

[*From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research*].

In a series of eight experiments dextrose was injected into dogs which had received 10 mg. of morphin per kilo of body weight. Ten other dogs received similar dextrose injections but no morphin, the slight operation having been performed under local anesthesia produced by cocain or ethylchloride. The dosage of dextrose was 4 gm. per kilo of body weight, injected in a 20 per cent. solution in about one hour. The difference in the urinary

and blood findings in these two series of experiments was quite striking. In the eight morphinized animals the average quantity of sugar in the urine secreted in two hours and a half (that is, from the beginning until one and a half hours after the end of the injection) amounted to 63 per cent. of the injected sugar, 80 per cent. being the largest and 50 per cent. the smallest quantity. The average quantity of sugar in the urine of six non-morphinized dogs in two hours and a half, amounted only to about 17 per cent. of the injected sugar, 30 per cent. being the highest and 4 per cent. the lowest quantity. There was, also, however, a difference between the two series of dogs in the volume of urine secreted. In the morphinized dogs the average amount of the injected sugar solution was 137 c.c. and of the urine 197 c.c.; in the non-morphinized dogs the average of the injected sugar solution was 187 c.c. and of the urine only 83 c.c. On this account experiments were made on four non-morphinized dogs in which the dextrose was dissolved in ¼ M solution of sodium sulphate, and there resulted a reversal in the relation of the volumes of the injected sugar and the urine: 212 c.c. of dextrose solution injected and 281 c.c. of urine secreted. Nevertheless, the elimination of sugar in the urine was not increased. In fact, in these four experiments the elimination of sugar in the urine was even less; it amounted on the average only to about 9 per cent. of the injected sugar, 13 per cent. being the highest and 7 per cent. the lowest quantity.

As to the sugar content of the blood, we may state briefly that in the non-morphinized dogs the original level was reached in half an hour after the end of the injection, while in the morphinized dogs that level was reached only one hour and a half after the end of the injection.

Summarizing briefly our results with regard to the effect of morphin we may say that, on the one hand, it increases considerably the elimination through the kidneys of intravenously injected dextrose, while, on the other hand, it perceptibly retards the return of the sugar content of the blood to its previous level.

# SCIENTIFIC PROCEEDINGS

ABSTRACTS OF COMMUNICATIONS.

**Seventy-sixth meeting.**

*Yale University, New Haven, May 24, 1916. Vice-President Gies in the chair.*

80 (1144)

## The therapeutic effect of wheat germ and of yeast in infantile scurvy.

By ALFRED F. HESS.

[*From the Bureau of Laboratories, Department of Health, New York.*]

As is well known, yeast is a specific therapeutic agent in the cure of beri beri or its prototype, polyneuritis gallinarum. Studies upon infants showed, however, that when autolyzed yeast was given in daily quantity of 15 to 30 cc. a day, it was unable to cure moderate cases of infantile scurvy, even when taken for a period of two to three weeks. Yeast was however able to bring about growth in infants.

Wheat germ was found to possess antiscorbutic power, which however cannot be compared to that of orange juice. In some instances it was able to prevent the occurrence of the subacute scurvy which follows the use of pasteurized milk; in one instance this disorder developed notwithstanding the fact that the infant had received wheat germ and the watery extract of the germ for many weeks.

Scurvy can develop while an infant is making steady gain in weight for weeks or months, and, on the other hand, the symptoms can disappear under antiscorbutic treatment, although no gain is manifested. It is therefore evident that growth is not an es-

sential factor connected with the scorbutic condition. This should be borne in mind, and the results of experiments on growth should not be considered as directly transferable to infantile scurvy or similar dietary diseases.

81 (1145)

## Oxygen utilization by fishes and other aquatic animals.[1]

By GEO. G. SCOTT.

[*From the United States Fisheries Biological Station, Woods Hole, Mass.*]

*A*. Lowering of the temperature causes a reduction in the rate of oxygen consumption. In one case, while one lot of fishes consumed 78 per cent. of the available oxygen supplied at 12° C; a similar lot of fishes in water 4° colder consumed but 60 per cent. In a second case, a fish in water at 20° C. consumed 94 per cent. of the oxygen present while a similar fish at 3° C. consumed but 57 per cent. Breathing had ceased in this case but was resumed on return to warmer water.

*B*. It was noted that oxygen was consumed more rapidly in tall, narrow vessels of water than in broad shallow ones. Fishes moving about in shallow vessels of water tend to reaerate the same. In one experiment to test this, one lot of fishes in tall narrow vessels of water consumed 80 per cent. of the oxygen supply, while in the broad shallow water at the end of the same period, the analysis indicated a reduction of but 20 per cent. of the original oxygen supply.

*C*. Fishes kept in dark vessels apparently consume oxygen at a less rapid rate than those exposed to light. Thus in the light one fish consumed oxygen at the rate of 0.12 c.c. per gm. per hr. while in the dark the rate was D. 11 c.c. per gm. per hr. But there was no evidence as to rate of oxygen consumption being less at night than in the daytime the rate being approximately the same.

*D*. Some forms show more resistance to low oxygen supply

[1] Published by permission of the Commissioner of Fisheries.

than others. This is particularly true of invertebrates. Respiration ceases altogether, and returns if the specimen is returned within certain time limits, to aerated water. The toad fish and killifish live in water with low oxygen content while butterfish and menhaden quickly succumb to reduction in oxygen supply.

*E.* The average rate of oxygen consumption for two species of marine worms is about 0.0205 c.c. per gm. per hr.; while that of two mollusks is about the same, *i. e.*, 0.0215 c.c. $O_2$ per gm. per hr. That of the fish, tautog, was 0.088 c.c. per gm. per hr. Most marine invertebrates consume oxygen at a very low rate; fishes at a much higher rate; with amphibia the rate is between that of invertebrates and fishes; the rate with mammals and birds is relatively high, that of birds being extremely high as compared with anatomically lower forms.

82 (1146)

## The nutritive value of some cotton-seed products in growth.

By THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

[*From the Laboratory of the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Connecticut.*]

When certain animals are fed on a ration containing an abundance of cotton-seed meal they frequently give evidence of so-called cotton-seed injury. This has been attributed to irritation from the indigestible husks, the oil, harmful microörganisms, and specifically toxic chemical compounds. The possibility suggests itself that the rations are frequently far from ideal or adequate in respect to the various essential nutrients, inorganic salts and "accessories." Richardson and Green[1] have found that when the ration of rats is otherwise suitable, toxic symptoms do not follow the use of cotton-seed meal. With their approval we refer to our own experiments, which are still in progress. To ascertain whether

[1] This has since been published: Richardson and Green, *Jour. Biol. Chem.*, June, 1916, XXV, 307.

the cotton-seed *proteins* are notably deficient for the purposes of nutrition, we have conducted feeding experiments on rats in which these proteins furnished practically all of the food nitrogen and in which the other essential dietary components were supplied by adding to the products to be tested a suitable mixture of "protein-free milk," butter fat and starch which, with the addition of adequate protein, has been shown in hundreds of experiments to be sufficient for perfect growth. In this way we have found that satisfactory growth can be made by rats when either cotton-seed globulin or the total cotton-seed protein precipitated from alkali extracts of cotton-seed meal is employed without other significant protein sources in the mixture. No toxic symptoms have appeared, even when the supposedly harmful *meal* also was used, during a period in which the animals reached a large size. In experiments in which the inorganic components were furnished by our "artificial protein-free milk" there was no failure of growth when the cotton-seed meal was used, thus suggesting that the latter contains the equivalent of the "determinant," "food accessory," or "vitamin" deemed essential for nutrition and furnished in fat-free milk. These results corroborate the conclusions of Richardson and Green[1] soon to be published.

83 (1147)

## The early responses of frog embryos to tactile stimulation.

By DAVENPORT HOOKER.

[*From the Anatomical Laboratory of the Yale University School of Medicine, New Haven, Conn.*]

In the course of some experiments on the regeneration of the spinal cord of frog embryos, it became necessary to establish certain facts in regard to their early tactile responses, as has been done for *Diemyctylus* and *Amblystoma* by Coghill. The results of this study are briefly summarized here.

The frog embryo exhibits a reaction toward the side stimulated as its first response to tactile stimulation with a fine human hair. This occurs so constantly that it must be regarded as normal for the frog, though only an occasional and aberrant reaction in the

[1] *Loc. cit.*

salamanders. This first response is followed by an avoiding-, a double-coil-, an S-reaction and the swimming movement, in order.

In a number of embryos, the cord was cut at different levels to determine the location in the cord where stimuli are transferred from one side of the body to the other. The results are, briefly, as follows: (1) when the cut passes through the brain, the portion anterior to the cut never responds to stimuli, while that posterior to it exhibits the usual series of reactions; (2) when the cut passes through the medulla, the same results are obtained; (3) when the cut passes through the middle of the body at a point just behind the medulla, both parts usually go through the normal series of reactions; (4) when the cut passes just anterior to the tail or (5) through the tail itself, the part of the body anterior to the cut goes through the normal series of responses, while that posterior to it remains negative. From these results it is evident that in the middle of the embryo there is a region about a millimeter in length which includes the upper part of the spinal cord and the lower part of the medulla, in which the decussations of the primary spinal nerve-paths take place, enabling the transfer of stimuli from one side of the body to the other.

A large series of experiments on this particular region show that the crossing does not take place at any one easily localizable point, but rather throughout the entire region. When the cord has been cut here, the two portions of the body thus isolated from each other go through the normal series of responses to stimuli independently of one another, the part in front of the cut usually being somewhat in advance of that behind it. Further, reversal of this region in no way affects the appearance of the responses, nor is it possible to differentiate between the time of appearance of reactions in the two extremities of the reversed piece.

Coghill suggests that the reaction toward the side stimulated, which appears as an aberrant form of response in *Amblystoma*, may be due to the transmission of the stimuli along the collaterals to the muscles of the same side before the main path to those of the opposite side is fully awakened. That this is actually the case in the frog is apparently demonstrated by the nature of the responses obtained as the embryo enters the second or avoiding-

reaction stage. At this time the embryos when first stimulated almost always give a reaction toward the side stimulated and only exhibit the avoiding reaction after several responses of the more primitive type. This would seem to indicate that connections across the body occur only as a summation of stimuli.

In conclusion, it may be stated that the early tactile responses in the frog embryo are very similar to those of *Amblystoma* except that they are preceded by a constant response toward the side stimulated. The localization of the decussation in the cord seems to cover a wider region than that described by Coghill for the salamander and in this region to appear simultaneously over a length of one half to one millimeter.

84 (1148)

## Permeability vs. tolerance of the kidneys for sugar in diabetes mellitus.

By ALBERT A. EPSTEIN.

[*From the Department of Physiological Chemistry, Mt. Sinai Hospital.*]

In the study of the relation of hyperglycemia to glycosuria in diabetic and non-diabetic conditions, the following facts have been elicited:

1. In diabetic individuals possessing healthy kidneys the glycosuria bears a definite relationship to the hyperglycemia.[1]

2. Cases of diabetes with definite renal disease, frequently show no relationship between the hyperglycemia and the glycosuria.[2] The hyperglycemia in such individuals is usually greater in proportion to the glycosuria than it is in those with normal kidneys. Means which promote renal secretion, increase the urinary output of sugar, with a consequent reduction of the hyperglycemia.

3. Acute impairment of renal function in clinical and experi-

[1] Epstein, Albert A., "Studies on Hyperglycemia in Relation to Glycosuria," Monograph, 1916, New York. PROC. SOC. EXPER. BIOL. AND MED., Vol. XIII, p. 67, 1916.

[2] Id.

mental diabetes leads to a diminution or cessation of the glycosuria with a progressive rise in the sugar content of the blood. The removal of both kidneys in animals, previously made diabetic by pancreatectomy, causes a progressive increase in the hyperglycemia.[1]

4. Operative procedures in non-diabetic individuals, involving the use of anesthetics (nitrous oxid and ether) lead to the development of a hyperglycemia, and rarely a glycosuria.

5. Cases of diabetes are frequently encountered showing no evidence of renal disease, in which the glycosuria disappears spontaneously or as the result of treatment, but in which a hyperglycemia persists. The hyperglycemia may be of high degree, and show slight or no variation.

When tests to ascertain the functional activity of the kidneys are instituted on the different types of cases represented above, the following phenomena are observed:

1. In diabetic individuals in whom the glycosuria is proportionate to the hyperglycemia the response of the kidneys to the phenolsulfonephthalein test is normal.

2. That when the hyperglycemia and the glycosuria in diabetic individuals do not show any relationship (the hyperglycemia being greater than one would expect to find with a limited glycosuria) there is a delayed excretion of phenolsulfonephthalein. This group of cases, as stated above, is usually demonstrably nephritic.

3. Non-diabetic cases, subjected to surgical procedures (under anesthesia) which develop a hyperglycemia but no glycosuria, show a delayed elimination of phenolsulfonephthalein.[2]

4. Diabetic individuals, who lose their glycosuria spontaneously or as a result of treatment, but retain a hyperglycemia, show a normal excretion of the dye.

5. From the observations thus accumulated, the following deductions are made:

1. When a diabetic process is active (as a result of disease or experimental procedures) actual disease or defective function of

[1] Epstein, Albert A., and Baehr, George, *J. Biol. Chem.*, Vol. XXIV, p. 1, 1916.

[2] Epstein, A. A., Reiss, J., and Branower, J., Soon to be published. Jour. Biol. Chem.

the kidneys leads to diminution or cessation of the glycosuria with a "progressive" accumulation of sugar in the blood. The hyperglycemia in such instances does not remain stationary, but rises steadily—and often very rapidly.

2. Surgical procedures (under anesthesia) cause a disturbance in the carbohydrate metabolism, with the consequent accumulation of sugar in the blood (hyperglycemia). A glycosuria in such cases is usually absent, evidently because the function of the kidneys is impaired.

3. Cases of diabetes which become "a-glycosuric" spontaneously or following treatment, retaining a hyperglycemia, reveal the fact that disturbance of renal function has no part in the process. Diminution or cessation of glycosuria through impairment of renal function leads, as a rule, to a progressive increase in the sugar content of the blood; but the glycosuria in these cases is not "progressive." The hyperglycemia in such cases may be of high degree, and remains uninfluenced by starvation. Furthermore, such of the cases as are relieved of their glycosuria by treatment, may upon liberal administration of carbohydrate, develop a glycosuria, with further increase in the hyperglycemia, and show a definite relationship between the two.

These facts are interpreted as signifying that a shifting in the plane of carbohydrate metabolism may take place, in diabetes so that the utilization of sugar by the tissues proceeds at a higher level. Whereas there is no a priori reason to believe that the utilization of sugar in the kidney differs in any way from that of any other organ or tissue, it is concluded, that renal permeability for sugar, is constituted of two phases: (1) a negative phase, *i. e.*, diminished permeability due to impairment of renal function; and (2) a positive phase, diminished permeability due to increased tolerance of the kidney for sugar.[1]

[1] Tests of renal function by means of lactose, according to Schlayer and Hedinger, are in progress, and the results will be reported later.

85 (1149)

## Studies in alimentary hyperglycemia and glycosuria.

By C. V. BAILEY.

[*From the Department of Medicine, New York Post-Graduate Medical School and Hospital, Dr. Edward Quintard, Director.*]

Using a modification of the Lewis and Benedict method[1] for the estimation of sugar in the blood the normal value seems to be between 0.09 and 0.12 per cent. Blood was examined in the morning before the patients had anything to eat or drink, the urine from a simultaneous half-hour period being tested for sugar.

Applying the above procedure it was found that in uncomplicated nephritis the blood sugar ranged from 0.12 per cent. in mild cases to 0.26 per cent. in severe cases with marked nitrogen retention. Cases of glycosuria upon admission were excreting anywhere from a mere trace to 6 or 7 per cent. sugar in the 24-hour specimen of urine. These cases seemed to fall into two distinct classes; (1)those having a normal or nearly normal morning blood sugar with urine sugar free by ordinary tests; (2) those having a high morning blood sugar (0.3 per cent. or over) and a comparatively small amount of sugar in the urine. In the former class were found the cases of "mild diabetes" and cases of hyperthyroidism; the latter class included cases with marked constitutional symptoms and definite signs of nephritis—"severe diabetes."

Tests of alimentary hyperglycemia and glycosuria were begun in the morning on an empty stomach. A specimen of blood and a half-hour specimen of urine were collected preceding the administration of a small quantity of glucose (30 to 90 grams in 400 c.c. weak tea). Following this the blood was tested at 15-minute intervals for the first 1½ hours and at ½ hour intervals for the succeeding 4½ hours. Half-hour specimens of urine were collected. The percentage of sugar was determined in the whole blood, plasma, unwashed corpuscles and urine. The units hemoglobin, percentage of corpuscles to whole blood and urinary se-

[1] Myers, V. C., and Bailey, C. V., *J. Biol. Chem.*, 1916, XXIV, 147.

cretion in cubic centimeters per minute were also determined in each specimen.

In an apparently normal subject the whole blood contained 0.12 per cent. glucose, the percentage in the plasma being slightly lower, and that in the unwashed corpuscles, slightly above that in the whole blood. The sugar in the urine was apparently about the same as in the plasma.[1] Following the ingestion of 75 grams glucose in 400 c.c. fluid the sugar in the blood rose evenly and rapidly, reaching its highest point in about 1 hour, returning to normal by the end of 2½ hours, falling below normal at the third hour, and from the fourth to the sixth hour retaining its normal level. The increase and decrease in the plasma seemed to be a little more rapid than in the whole blood, although the difference was very slight. The hemoglobin dropped 3 to 5 per cent. in from 15 to 70 minutes, then increased rapidly, later more slowly, reaching its normal in from 1½ to 3 hours. Urinary secretion decreased during the development of the hyperglycemia, increasing as the blood sugar decreased. The sugar in the urine apparently increased at the same rate as in the blood up to a concentration of 0.17 per cent. From this point the increase was much more rapid in the urine, so that when the blood sugar had reached its highest point, 0.23 per cent. at the end of one hour, the urine contained 0.9 per cent. sugar. The decrease in the urine sugar was rapid for the succeeding hour, then much slower, so that the normal concentration was not reached until about 6 hours after the ingestion of the sugar.

In a case of renal diabetes there was an initial hypoglycemia with a marked glycosuria (3 per cent.). The blood sugar curve was of the normal type, but the urine sugar curve abnormally high.

In a case of early diabetes the initial blood sugar and urine sugar values were normal. Alimentary hyperglycemia was rapid, the highest point being reached in about one-half hour, return to normal taking place in less than 2 hours. The urine sugar curve was abnormally high with a sluggish return to normal.

In diabetes of long standing without signs of nephritis, the initial blood sugar value was high (0.2 per cent.), the urine value normal. Blood sugar and urine sugar curves were of the previous

[1] See Myers, V. C., PROC. SOC. EXPER. BIOL. AND MED., 1916, XIII, 180.

type, but the blood sugar curve was higher and of longer duration.

Cases of diabetes with signs of nephritis showed an initial high blood sugar with comparatively low urine sugar. The blood sugar curve increased at about the normal rate but return to normal did not take place before 4½ to 6 hours. The urine sugar curve was low, the highest concentration being 1.5 per cent., although the blood at that time contained 0.31 per cent. sugar.

Cases of chronic nephritis showed an initial high blood sugar, 0.16 per cent., with urine normal. Alimentary hyperglycemia was delayed and prolonged, the highest point being reached in 2 hours and return to normal not taking place before 4 to 6 hours. The highest point in the urine sugar curve was 0.5 per cent., the blood at that time containing 0.37 per cent.

A case of chronic parenchymatous nephritis showed a constant glycosuria of 0.5 per cent. This was independent of the blood sugar up to the latter's concentration of 0.21 per cent. In a second test where the blood sugar reached 0.4 per cent. the urine sugar increased to 1.0 per cent., later decreasing and continuing at 0.5 per cent., the blood containing 0.2 per cent.

Cases of myxedema and hypopituitarism were also studied. In these cases the initial blood sugar and urine sugar values were normal. Alimentary hyperglycemia was delayed and prolonged as in nephritis and kidney permeability was greatly decreased.

86 (1150)

## The digestibility and utilization of egg-proteins.

By **W. G. BATEMAN.** (By invitation.)

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Connecticut.*]

Raw egg-white is found to be a decidedly indigestible substance. It may cause diarrhea in dogs, rats, rabbits and men when ingested in any large quantity. Its utilization by the body is poor since it is used only to the extent of from 50 to 70 per cent. Subjects can acquire a certain tolerance for the native protein after ingesting it for several days so that it no longer causes diarrhea and is somewhat better utilized.

Raw egg-white can be made digestible through coagulation by heat; by precipitation with alcohol, chloroform, or ether; by incubation with dilute acids or alkalies; by partial digestion by pepsin; by conversion into alkali meta-protein.

The indigestibility of native egg-white probably lies either in its antitryptic content or in its chemical constitution. Its physical texture appears to play a minor part in its behavior.

Of the individual proteins constituting egg-white, the albumin fraction appears to be the indigestible component.

The whites of the hen's egg and duck's egg act alike in causing diarrhea and in being poorly utilized.

Egg-yolk either raw or cooked is excellently utilized. It sometimes causes digestive disturbances in dogs, apparently because of its high fat content.

A review of the literature shows that dietitians have relied, in general, upon the early observations of Beaumont as support for the use of raw eggs. These observations were in the main exact; but, so far as the digestibility of raw egg-white is concerned, were misinterpreted.

In current dieto-therapy raw whole eggs, raw egg-white and albumen-water are extensively prescribed. There appears to be little in their conduct as foodstuffs, however, to warrant such faith in their nutritive value or ease of assimilation.

87 (1151)

## The position of the head after experimental removal of the otic labyrinth.

By **A. L. PRINCE.** (By invitation.)

[*From the Physiological Laboratories of Columbia University, and the Yale Medical School.*]

In the vertebrates usually employed in the physiological laboratory, unilateral destruction of the otic labyrinth is immediately followed by a permanent torsion of the head to the injured side.[1] In a series of experiments on cats, I have found that this posture is associated with diminished tonus in the cervical

[1] Wilson and Pike, *Philosophical Transactions of the Royal Society*, London, 1912, series B, Vol. 203, pp. 127–160.

musculature on the side of the lesion. Although this investigation is as yet incomplete, it is considered desirable to report the following facts.

As a means of determining the effect of impaired tonus on torsion of the head, apart from that arising from destruction of the labyrinth, the following procedure was adopted:

1. UNILATERAL SECTION OF THE DORSAL ROOTS OF THE CERVICAL NERVES.

Accompanying the impairment of muscular tonus occasioned by this procedure, there is torsion of the head to the side of the injury. This torsion can only be attributed to the unbalanced activity of the neck muscles on the intact side. It is to be noted that the character of torsion following section of the dorsal roots of the cervical nerves does not differ greatly from that seen after unilateral removal of the labyrinth. In a dog, which has not yet come to autopsy, section of the dorsal roots was followed by torsion of the head to the side away from the lesion.

The remaining series illustrate the torsional effect of various combined lesions.

2. UNILATERAL REMOVAL OF THE LABYRINTH AND SECTION OF THE DORSAL ROOTS OF THE CERVICAL NERVES ON THE OPPOSITE SIDE.

As stated before, unilateral removal of the labyrinth is followed by torsion of the head to the side of the injury. Upon subsequent section of the dorsal roots of the cervical nerves on the opposite side this torsion is greatly reduced and in some cases entirely disappears. Reversal in the order of the experiment does not affect the end result; the torsion resulting from the first procedure is always decreased or abolished by the second.

3. UNILATERAL REMOVAL OF THE LABYRINTH AND SECTION OF THE DORSAL ROOTS OF THE CERVICAL NERVES ON THE OPPOSITE SIDE FOLLOWED BY EITHER (*a*) REMOVAL OF THE REMAINING LABYRINTH, OR (*b*) SECTION OF THE REMAINING DORSAL CERVICAL ROOTS.

In these experiments the final procedure is followed by a reappearance of the head torsion. The direction of the torsion, however, is always to the side on which two lesions are combined.

4. UNILATERAL REMOVAL OF THE LABYRINTH AND SECTION OF THE DORSAL ROOTS OF THE CERVICAL NERVES ON THE SAME SIDE.

When these procedures are successively applied to the same side, the degree of head torsion brought on by the first procedure is always accentuated upon application of the second. As in the experiments of the series 2, the order of the experiment can be reversed without influencing the results.

5. UNILATERAL REMOVAL OF THE LABYRINTH AND SECTION OF THE DORSAL ROOTS OF THE CERVICAL NERVES ON THE SAME SIDE, FOLLOWED BY EITHER (*a*) REMOVAL OF THE REMAINING LABYRINTH, OR (*b*) SECTION OF THE REMAINING DORSAL CERVICAL ROOTS.

In this series the accentuated torsion appearing after the second procedure is decreased on applying the third.

These results indicate that the torsion of the head resulting from destruction of the labyrinth is caused by an impairment of tonus in the neck muscles on the side of the lesion. The cervical musculature involved in torsion of the head is influenced by two distinct tonus mechanisms. The afferent impulses of the first mechanism arise in the otic labyrinth; those of the second arise in the peripheral endings of the fibers of the dorsal roots of the cervical nerves. Injury to either of these two mechanisms does not result in absolute loss of tonus in the cervical musculature, for when destruction of the labyrinth and section of the dorsal roots of the cervical nerves are combined on the same side, the degree of head torsion brought by the first lesion is somewhat accentuated by the second.

The relation of possible cerebellar paths to the labyrinthine head torsion is now under investigation. The data available at present are outlined below.

6. UNILATERAL REMOVAL OF THE LABYRINTH AND SECTION OF THE INFERIOR CEREBELLAR PEDUNCLE ON EITHER SIDE.

The torsion resulting from destruction of the labyrinth is not modified to any considerable extent by section of the posterior cerebellar peduncles.

## Conclusions.

The torsion of the head after unilateral removal of the labyrinth is due to the preponderating activity of the muscles of the intact side. The afferent impulses concerned come largely from the labyrinth, the muscles, the tendons of the neck, and the articulations of the cervical vertebræ.

88 (1152)

## Is uterine activity subject to cerebral control?

By **H. G. Barbour** and **N. H. Copenhaver.** (By invitation.)

[*From the Department of Pharmacology, School of Medicine, Yale University.*]

Although morphin is known to delay the progress of labor we have hitherto been unable to detect any inhibitory influence of this drug upon the tone or activity of the uterus in animals. It causes rather an increase in tone in the isolated uterus of cat and guinea pig,[1] and often in the intact uterus of the decerebrate cat or anesthetized rabbit.[2] The only inhibition of the uterus by morphin which we have observed previous to the present work has been accounted for by circulatory collapse.

Conditions of anesthesia or decerebration under which the morphin was given in our previous work have, by exclusion, led us to the belief that morphin, in clinical doses, inhibits uterine activity by a purely cerebral action. Desiring more direct evidence on this point we were led to inquire into the nature of cerebral control of the uterus, if any exists.

To this end we have begun by the employment of a method subjecting a part of the cortex and basal ganglia to the influence of cold and heat. This is done by means of a double metal tube fixed in the skull of a rabbit, on one side, anterior to the coronal suture and passing through the anterior portion of the corpus striatum to the base of the skull. The lateral ventricle is usually entered. This procedure, which was first employed by one of us

[1] Barbour, H. G., and Copenhaver, N. H., *Journ. Pharm. and Exp. Ther.*, 1915, VII, 529.

[2] Barbour, H. G., *Journ. Pharm. and Exp. Ther.*, 1915, VII, 547.

in the study of cerebral control of body temperature,[1] is performed aseptically under light ether anesthesia. As soon as the animal is free from the narcotic certain cerebral functions may be influenced by the passage through the tube of hot or cold water.

The advantages of such a method are (1) the confinement of the effective agent entirely to the cerebrum (or a portion of it), (2) the absence of an anesthetic, and (3) the ease with which the functional activity of the brain can be quickly altered in either of two opposite directions.

The uterine activity was recorded by air conduction from a finger cot inflated within the rabbit's uterus. Most of the animals used were in early pregnancy. The results obtained by this method show that under a few minutes of cerebral cooling (10° C.) the cavity of the uterus becomes much diminished in volume and there is a tendency to an increase in the amplitude and frequency of the individual contractions; on the other hand a change to heating (45° C.) soon causes a reversion to original conditions. Although voluntary limb movements are sometimes a disturbing factor we have been able to exclude these entirely as the cause of the changes described.

There is however no doubt that the changes in volume of the uterine cavity are largely dependent upon changes in tone of the abdominal musculature. One can readily follow with the hand the contraction and relaxation of the recti, for example, which are associated respectively with cooling and heating of the cerebrum. Furthermore the uterine changes were not observed in two curarized animals, nor were they obtainable in an animal with cord completely transected between the sixth and seventh dorsal vertebræ. However, under both of the latter conditions the normal activity of the uterus was very feeble.

The method of excluding the voluntary abdominal muscles by suspending the intact uterus, surrounded by warm oil, in a cylinder has failed to give very positive evidence of a direct cerebral control of the uterus. This method has always been pursued under light anesthesia however. In one of six animals there was under cerebral cooling a marked increase in tone which was not diminished by cessation of the cooling process. In another the

[1] Barbour, H. G., *Arch. exp. Path. u. Pharm.*, 1912, 70, 1.

amplitude of the individual contractions increased markedly under cooling and diminished under heating. The other four experiments were negative.

Thus far then we have established a definite cerebral influence over the volume of the uterine cavity. The fact that this appears to be largely if not entirely a control of the voluntary musculature of the abdomen does not detract from its importance in connection with the birth process.

Returning to the morphin question, we have now given small subcutaneous doses of this substance in two animals which had responded well to cerebral cooling and heating in the manner above described. Here the morphin, given to unanesthetized animals, resulted in a depression of the uterine activity, although the dose was so small in one case (.01 gram per kilo) that the animal remained sitting upright and occasional normal limb movements continued to occur. Cerebral cooling now had no effect upon the volume of the uterine cavity of these morphinized animals, showing clearly how morphin can influence labor by a central action.

89 (1153)

## Endomixis in diverse races of Paramaecium aurelia.

By LORANDE LOSS WOODRUFF.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

Woodruff and Erdmann in 1914[1] described a normal periodic reorganization process without cell fusion, which they termed endomixis, in *Paramaecium aurelia*. This study was based chiefly on pedigreed cells from Woodruff's 5,000-generation race of *Paramaecium aurelia*, though specimens of a race of this organism isolated by Erdmann in Germany showed the same phenomenon.

The present communication is to prove the general occurrence of endomixis in races of *Paramaecium aurelia*, since this has been questioned, on a priori grounds, by certain authors.

The following races of *Paramaecium aurelia*, in addition to those mentioned above, have now been studied:

Oberlin Race. Isolated at Oberlin, Ohio. Carried in pedi-

[1] *Loc. cit.*

greed culture from October 8, 1914, to date, during which time it has attained 951 generations.

Bryn Mawr Race. Isolated at Bryn Mawr, Pa. In pedigreed culture from January 7, 1915, to February 8, 1916, when it was discontinued at the 650th generation.

Oxford Race. Isolated at Oxford, Ohio. Pedigreed culture started on July 16, 1915, and has to-day (May 24, 1916) attained the 779th generation.

Woods Hole Race. Isolated at Woods Hole, Mass. Pedigreed culture begun on August 11, 1915, and discontinued on January 14, 1916, at the 305th generation.

Each of the above races has shown endomixis at the regular rhythmic periods throughout its culture and therefore this additional data from races from diverse sources fully corroborates the statement of Woodruff and Erdmann[1] that "this reorganization process is a normal phenomenon and probably occurs in all races of the species *Paramaecium aurelia*."

90 (1154)

## Further investigations on the cyclic changes in the mammalian ovary.[2]

By LEO LOEB.

[*From the Department of Comparative Pathology, Washington University, St. Louis, Mo.*]

In former investigations I have described cyclic changes in the ovaries of the guinea pig which depend largely upon injurious influences exerted upon the ovaries in the period directly pre-

[1] Woodruff and Erdmann, "Complete Periodic Nuclear Reorganization without Cell Fusion in a Pedigreed Race of *Paramaecium*," PROC. SOC. FOR EXPER. BIOLOGY AND MED., Vol. 11, 1914 (preliminary paper). Erdmann and Woodruff, "Vollstandige periodische Erneuerung des Kernapparates ohne Zellverschmelzung bei reinlinigen Paramaecien," *Biol. Centr.*, Bd. 34, 1914 (preliminary paper). Woodruff and Erdmann, "A Normal Periodic Reorganization Process without Cell Fusion in *Paramaecium*," *Journal of Exper. Zoology*, Vol. 17, No. 4, 1914 (complete paper).

[2] During the summer 1915 serial sections of a number of ovaries were made for me in the department of anatomy of Washington University. I wish to express my appreciation to Dr. R. T. Terry for placing the facilities of his laboratory at my disposal.

ceding ovulation. Inasmuch as ovulation depends upon degenerative changes having previously taken place in the corpora lutea, the cyclic changes in the life of the follicles are correlated with the cyclic changes in the corpus luteum. It was our aim to determine whether the same ovarian cycle existed in all mammalian ovaries; we examined for this purpose ovaries of the rabbit and of the ferret at various periods of sexual activity.

Summarizing our observations we may state that neither in the rabbit nor in the ferret do cyclic changes in the follicular apparatus of the ovaries, comparable to those of the guinea pig, occur. In the period immediately preceding or following ovulation no marked degeneration of the follicles takes place. If any follicles degenerate at all (in consequence of the circulatory changes in the ovaries during this period?), such a degeneration can only affect a few large follicles, while in the guinea pig a sudden degeneration of all the follicles, with the exception of the smallest ones, takes place during this period. The other changes subsequent to this sudden disintegration of follicles in the guinea pig are likewise absent in the rabbit and ferret.

The ovaries of the guinea pig also differ in other respects from those of the rabbit and ferret:

1. In the guinea pig a so-called "interstitial gland" is absent, while it is present in the ovaries of the rabbit and ferret.

2. In the guinea pig during heat a spontaneous ovulation usually takes place. This ovulation in no way depends upon a preceding copulation; while in the rabbit and, as far as we could determine, also in the ferret, heat as such is not sufficient, but a copulation needs to take place in order to insure ovulation.

It would be of interest to determine whether there exists a general correspondence of these various factors in such a way that animals which, like the guinea pig, do not have an interstitial gland and ovulate "spontaneously," show a very marked cycle in the ovarian follicular apparatus, while animals that possess an interstitial gland and do not ovulate "spontaneously" do not possess such a cycle.

It shall be determined in subsequent investigations whether cyclic or other changes occur in the interstitial gland of rabbit and ferret. We may, however, state here that during the winter

period (December and first half of February) the ovaries of the ferret are small and differ from the ovaries in the period of sexual activity especially through the diminution in the number of good follicles. While the ovaries as a whole are smaller, the interstitial gland is well preserved during the winter months.

91 (1155)

## The cyclic changes in the mammary gland of the guinea pig.

By CORA HESSELBERG and LEO LOEB.

[*From the Department of Comparative Pathology, Washington University, St. Louis, Mo.*]

Our interest in the character of the cyclic changes of the mammary gland and in their mechanism was twofold. (1) In former investigations Loeb has shown that an early extirpation of the ovaries reduces to a very marked extent the incidence of cancer of the breast in mice. It was therefore of interest to inquire more closely into the relations between ovaries and mammary gland, and (2) we wished to determine whether there exists a parallelism between the cyclic changes in the mammary gland on the one hand and in the ovaries and uterus on the other hand. One of us had formerly shown that in the cyclic changes of ovaries and uterus we could distinguish two phases: the first, comprising ovulation and the heat changes in the uterus, depends upon the absence of the corpus luteum. These are prevented by a substance secreted by the lutein cells. This phase is, however, dependent upon another constituent of the ovaries. The second phase, comprising the further cyclic changes in the uterus as well as the production of decidua and deciduomata, requires a substance secreted by the corpus luteum. Do corresponding phases exist in the case of the mammary gland?

Relatively little is known concerning the cyclic changes in the mammary gland. Bouin and Ancel, as well as Frank and Unger, have shown that in the rabbit, even in the absence of pregnancy, but in the presence of corpora lutea, proliferation takes place in the mammary gland. Proliferation also occurs regularly during pregnancy. Frank and Unger have furthermore demonstrated

that the experiments of Starling and others concerning the source of the growth substance which acts on the mammary gland are not conclusive.

Our investigations concern the cyclic changes in the mammary gland of the guinea pig. We studied the mammary glands as well as uterus and ovaries in 262 animals, in almost all of which the time of ovulation had been ascertained prior to the experiment. In many of these animals the effect of ovaries and uterus on the cyclic changes was analyzed by various experimental procedures.

Without going into a detailed discussion of our results, we may state our principal conclusion as follows: The normal sexual cycle of the guinea pig (the period between two ovulations), has a duration of approximately 16–18 days. We can also, in the case of the mammary gland, distinguish two phases in this cycle—one comprising the time of heat and ovulation and two or three days following ovulation; in the large majority of cases the mammary gland proliferates mitotically during this phase. In the second phase, comprising the remainder of the sexual cycle, proliferation is as a rule absent. Only toward the end of this phase, from the fifteenth to the twentieth day, we find again in some cases proliferation. The first proliferating phase depends upon the absence of the corpus luteum. We can accelerate it by an early extirpation of the corpora lutea, in a way similar to the acceleration of ovulation and uterine heat changes by the same procedure. The corpus luteum of the ordinary sexual period in the guinea pig does not usually produce proliferation of the mammary gland. Also during pregnancy, which lasts in the guinea pig about twice as long as in the rabbit, proliferation of the mammary gland occurs regularly only after the twenty-fourth day of pregnancy.

If through certain experimental procedures we prolong the sexual cycle, we find usually a proliferating gland in cases in which well-developed living deciduomata and good corpora lutea, or in which strongly developed, not degenerated corpora lutea without deciduomata, are present. In those cases in which during the period of prolongation deciduomata and corpora lutea are degenerating, proliferation of the mammary gland as a rule is absent.

In case of castration and of the presence of hypotypical ovaries, proliferation of the mammary gland is not found. Con-

sidering all the facts we may conclude that while proliferation in the first phase depends upon the absence of the corpus luteum and upon the activity of another constituent of the ovaries, the proliferation which is found following the first period is in all probability due to substances secreted by the corpus luteum.[1] In the guinea pig, however, the effect of this substance becomes apparent only at a much later period than in the rabbit. The adaptive character of this phenomenon is clear if we remember that in the rabbit the functioning of the mammary gland is required at a much earlier period than in the guinea pig. Repeated intraperitoneal injections of corpus luteum of the cow does not produce a proliferation of the mammary gland in the guinea pig.

92 (1156)

## The chlorides of the plasma in uremia.

By FRANKLIN C. MCLEAN. (By invitation.)

[*From the Hospital of the Rockefeller Institute for Medical Research, New York.*]

Previous investigations relating to the chlorid content of the blood or plasma in uremia have yielded conflicting results, some figures much lower than the lowest normal limit having been reported.[2] It is known that the chlorid content of nephritic plasma is usually somewhat higher than that of the average normal plasma, but the findings in uremia have apparently so far not been explained.

We have been able, in several cases, to make frequent observations of the chlorid content of the plasma of nephritic individuals during life, and up to the time of death in uremic coma. We have found a diminution of chlorids in the plasma to be the usual accompaniment of uremia, and we have found this decrease of chlorids in the plasma to accompany the increased H + ion concentration frequently observed in the blood of uremic patients shortly before death.

[1] The latter part of this conclusion depends in part at least upon the correctness of the observations of Bouin and Ancel and Frank and Unger.

[2] Strauss, H., "Die chronischen thieren entzündungen," Berlin, 1902, 51.

That increased acidity of the blood causes a diminution of the plasma chlorids, and an increase in the chlorid content of the cells had been shown experimentally both in vitro and in vivo by Hamburger.[1] That a similar change occurs with the increased acidity of the blood in uremia is illustrated by the following case of nephritis, terminating in uremia.

*Case 1.* P. W. M., male, age 44, chronic interstitial nephritis, uremia. Patient admitted June 17, suffering with chronic interstitial nephritis, hypertension and secondary cardiac failure with edema. With rest in bed the heart condition rapidly improved and the edema disappeared. Following this the patient felt well and the condition remained stationary, until October 12, when an impending uremia first became manifest by an increase in the blood urea and a diminished urea excretion. From June 17 to October 6 there were made twenty blood analyses, with simultaneous urine analyses, and the results showed very slight variation. The average of the figures for this period is shown below. The maximum concentration of NaCl in the plasma during this period was 6.53 grams per liter and the minimum was 6.06. The phthalein elimination, shortly after admission, was twice found to be 8 per cent. in two hours.[2]

It will be seen from the table that the diminished urea function, beginning about two weeks before symptoms of uremia appeared, was accompanied by a diminution in the reserve alkalinity of the plasma, as shown by Van Slyke's method. On October 29 an actual increase in acidity, as shown by the $P_H$ of the blood, was present and there had been a sudden fall in the chlorid concentration of the plasma. This concentration remained low thereafter until death, and the change was far greater than could possibly be accounted for by the diminished diet. The change in NaCl concentration in the plasma is exactly that produced by intravenous injection of any acid, in quantities sufficient to change the $P_H$ of the blood, and is accounted for by the increased acidity of the blood occurring during the disease.

[1] Hamburger, H., "Osmotischer Druck und Ionenlehre," Wiesbaden, 1902, I, 317.

[2] The observations on urea and chlorid elimination were made by the methods previously described by the author (*Jour. Exper. Med.*, 1915, XXII, 212 and 366). Plasma $CO_2$ was determined by the method of Van Slyke (PROC. SOC. EXP. BIOL. AND MED., 1915, XII, 165). For the determination of hydrogen ion concentration in the whole blood by the gas chain method, I am indebted to Mr. G. E. Cullen.

TABLE I.

| Date, 1915. | Hour. | Blood Urea per Liter, Gm. | Index of Urea Excretion. | Plasma Sodium Chlorid per Liter. | | | Plasma $CO_2$ by Volume, %. | H+ Ion Concentration Whole Blood $P_H$. | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Calculated, Gm. | Actual, Gm. | Difference, Gm. | | | |
| June 17 to Oct. 6 | | 1.176 | 5.5 | 5.86 | 6.27 | +0.41 | 49.1 | | |
| Oct. 12 | | 1.542 | 2.9 | 5.86 | 6.25 | +0.39 | | | Patient feels quite well |
| Oct. 21 | | 2.125 | 1.5 | 5.92 | 6.34 | +0.42 | | | Appetite slightly diminished |
| Oct. 26 | | 3.005 | 0.7 | 5.84 | 6.28 | +0.42 | 34.8 | | Complains of headache and muscular cramps |
| Oct. 27 | | 3.285 | 0.4 | 5.84 | 5.89 | +0.05 | 19.0 | | Nausea and vomiting |
| Oct. 28 | | 3.430 | 0.27 | 5.78 | 5.83 | +0.05 | 20.9 | | Severe headache; typical air hunger |
| Oct. 29 | | 3.935 | 0.17 | 5.73 | 5.02 | −0.71 | 17.6 | 6.86 | Headache; stertorous breathing; mental condition good |
| Oct. 30 | 9 A.M. | 4.580 | | | 5.53 | | 15.2 | 6.93 | Partial coma; muscle twitchings |
| | 11:45 " | | | | 5.75 | | 12.2 | | |
| | 1-15 P.M. | | | | 5.30 | | 32.8 | 7.44 | After $NaHCO_3$, 30 gms. intravenously |
| | 2:30 " | 4.620 | | | 5.69 | | 18.7 | 7.29 | |
| | 9 " | | | | 5.46 | | 21.4 | | |
| | 9:30 " | | | | 5.32 | | 39.4 | | After $NaHCO_3$, 25 gms. intravenously |
| Oct. 31 | 10 " | 5.020 | | | 5.31 | | 33.3 | | Deep coma |
| Nov. 1 | | 5.746 | | | | | | | Died |

Intravenous administration of sodium bicarbonate brought back the reaction of the blood temporarily to a normal point of 7.44 without influencing the course of the uremia. Both injections caused a still further lowering of the chlorids in the plasma. An identical effect is produced by intravenous administration of any salt and must be considered a salt action, rather than an alkali effect, as injection of strong alkalies increase the concentration of chlorids in the plasma.[1]

[1] Hamburger, H., loc. cit.

93 (1157)

## The stimulating influence of alkali on hepatic glycogenesis.

By J. J. R. MACLEOD.

[*From the Physiological Laboratory, Western Reserve Medical School, Cleveland, O.*]

The pronounced influence which acids and alkalies exert on the rate at which glycogenase converts glycogen into reducing sugar has suggested the possibility that variations in the activity of this enzyme in the liver cells may depend primarily on changes occurring in the reaction (H-ion concentration) of the immediate environment in which the enzyme is acting.[1] Although there are now many facts which indicate that a condition of hyperglycemia (and glycosuria) usually develops when acids gain entry to the blood and that the opposite condition of hypoglycemia is readily induced with alkalies, yet there is no direct evidence that these changes in the reducing power of the blood are immediately dependent upon corresponding alterations in the rate of hepatic glycogenolysis in warm blooded animals.

A direct method for testing the influence of changes in reaction in the liver cells on the glycogenolytic process is offered in the experimental procedure which has recently been described by Pearce and Macleod.[2] Briefly, this consists in an estimation of the reducing power of the blood removed at short intervals (2–3 minutes) from the portal vein and vena cava inferior (opposite the entry of the hepatic veins), several consecutive estimations being made before, during and after the injection of a dextrose solution under constant pressure, into a branch of the mesenteric vein. When the percentage reducing power is equal in the two bloods, the glycogenolytic process in the liver is presumably dormant; when the percentage is higher in the vena cava than in the portal vein, glycogenolysis must be active; and when lower,

[1] Cf. Pavy and Bywaters, *Journ. Physiol.*, 1910, LXI, p. 168; Elias, *Biochem. Ztschr.*, 1913, XLVIII, p. 120; Elias and Kolb, ibid., 1913, LII, p. 331; Macleod, "Diabetes, etc.," 1913, p. 150; Murlin and Kramer, *Jour. Biol. Chem.*, 1913, XV, p. 365; and *Proc. Soc. Biol. Chem.*, 1915, III, p. 25, and Kramer and Marker, ibid., p. 24.

[2] Macleod and Pearce, *Am. Journ. Physiol.*, 1915, XXXVIII, p. 425.

the opposite (namely, a building up of glycogen out of the injected sugar) must be taking place. In previous investigations, in which neutral solutions were employed, no retention of dextrose by the liver could be demonstrated when about 0.5 gm. was injected into the portal circulation during five minutes.

In the present investigation, the injected dextrose solution was either faintly acid or strongly alkaline, the latter reaction being obtained by adding from 5 to 20 gm. $Na_2CO_3$ (anhydrous) to 120 c.c. of the solution. At the rates of injection employed, a distinct change occurred in the H-ion concentration of the blood of the portal vein, but much less so in that of the vena cava, as judged by the dialysis-colorimetric method of Levy, Rowntree and Marriott.

In most of the experiments the reducing power of the blood was determined by the method of Lewis and Benedict as modified by R. G. Pearce. In two experiments the Bertrand method was employed after precipitation of the proteins by colloidal iron. The following table depicts some of the most typical results.

TABLE

AVERAGE PER CENT. REDUCING POWER OF BLOOD

| No. | Before Injection. | | During Injection. | | Amount of Dextrose Injected in 5 Minutes. |
|---|---|---|---|---|---|
| | In Portal Vein. | In Vena Cava. | In Portal Vein. | In Vena Cava. | |
| 19 | 0.070 | 0.068 | 0.153 | 0.104 | 1.6 gm. |
| 20 | 0.067 | 0.072 | 0.098 | 0.086 | 1.35 " |
| 25 | 0.101 | 0.102 | 0.190 | 0.129 | 0.51 " |
| 32 | 0.086 | 0.088 | 0.208 | 0.127 | 0.50 " |
| 27 | —— | 0.126 | 0.209 | 0.158 | 0.48 " |
| 30 | —— | 0.125 | 0.196 | 0.100 | 0.36 " |

In Experiments 19, 20, 25, and 31 the picric acid method was employed, and in 27 and 30 that of Bertrand.

Control experiments in which the dextrose solution was made faintly acid, or contained an excess of sodium chloride (16 per cent.) did not reveal any such difference in the reducing power of the two bloods. Neither did injections of acid or alkali alone cause any difference. Many other details remain to be further investigated. For the present, however, the results clearly demonstrate that, when dextrose is injected in moderate amounts into the

blood of the portal system, a large proportion of it becomes retained in the liver provided alkali is simultaneously injected in sufficient amount to produce a distinct lowering of the H-ion concentration of the portal blood. A similar retention can not be demonstrated by the above method when the dextrose solution is neutral or acid, or when it is made markedly hypertonic with sodium chloride.

94 (1158)

## Endothelial opsonins.

By W. H. MANWARING and HARRY C. COE.

[*From the Department of Bacteriology and Immunity, Leland Stanford, Jr., University.*]

If the blood-free liver of a normal rabbit is repeatedly perfused with a sample of Ringer's solution containing a known number of pneumococci, no diminution in the pneumococcic count of the perfusion fluid is observed, even after a dozen passages through the liver.

If the liver of an actively immunized rabbit is similarly perfused, the pneumococcic count is rapidly decreased. After three or four passages, the perfusion fluid usually becomes sterile.

Histological study of the perfused liver now shows numerous pneumococci adherent to the capillary endothelium. Few if any agglutinated masses are seen.

Normal rabbit serum added to the perfusion fluid in amounts not exceeding 10 per cent. causes no appreciable retention of the pneumococci by normal livers. Immune serum similarly added causes a quantitive retention of the pneumoccoci.

Immune serum will cause this retention when tested in less than a hundredth of the concentration necessary to cause agglutination.

The serum component causing the pneumococcic retention is thermo-stable (60° C., 30 min.).

Unagglutinated pneumococci sensitized by exposure to immune serum and then washed free from serum, are retained quantitatively by normal livers.

The serum component responsible for the retention is therefore

evidently an opsonin or bacterio-tropin so altering the pneumococci as to cause their adhesion to the capillary walls.

This opsonin is relatively inactive for the extrahepatic capillaries. The hind-quarters, lungs, kidney and intestines of normal rabbits can be repeatedly perfused with Ringer's solution containing as much as 1 per cent. immune serum, with only a slight retention of the pneumococci by these organs, while 0.001 per cent. immune serum will cause their quantitative retention by the liver. (Spleen and bone-marrow not yet tested.)

Defibrinated normal rabbit blood used as the perfusion fluid will cause a slight deposit of the pneumococci in all organs.

95 (1159)

## Specific receptors of fixed tissues.

By W. H. MANWARING and YOSHIO KUSAMA.

[*From the Department of Bacteriology and Immunity, Leland Stanford, Jr., University.*]

If Ringer's solution containing 1 per cent. goat serum is repeatedly perfused through the blood-free liver of a normal, anaphylactic or immune rabbit, no diminution in the amount of goat serum in the perfusion fluid is produced, that can be detected by titration with a specific precipitating serum.

If defibrinated normal, anaphylactic or immune rabbit blood is added to the perfusion fluid, diminutions in the amount of goat serum are observed after repeated liver passage; but in all cases these diminutions are identical with diminutions observed in control samples of the fluid kept at incubator temperature and not passed through the liver.

The perfusion experiments therefore furnish no evidence of the existence of a specific receptor apparatus (Ehrlich) for goat proteins, in normal, anaphylactic, or immune rabbit livers.

96 (1160)

## Protein absorption by blood corpuscles.

By W. H. MANWARING and YOSHIO KUSAMA.

[*From the Department of Bacteriology and Immunity, Leland Stanford, Jr., University.*]

If 1 per cent. goat serum is added to freshly drawn defibrinated normal rabbit blood, the mixture incubated for one hour, and then separated by centrifugation into serum and corpuscle fractions, a titration of the serum fraction by specific precipitin methods will usually show but 25 per cent. of the goat protein originally added to the blood.

If the serum and corpuscle fractions so obtained are allowed to undergo independent autolysis (10 hrs., 37° C.), a distinct restoration of the goat protein is observed in each fraction. The restoration of the protein in the corpuscle fraction, however, is usually much more pronounced than that in the serum fraction, and may amount to as much as 50 per cent. of the total protein originally added to the blood.

If goat serum is slowly injected intravenously into normal rabbits in amounts not exceeding 1 per cent. of the total blood volume, and blood is withdrawn from 1 to 4 hours later, a distinct restoration of the goat protein can be brought about by allowing the centrifuged but unwashed corpuscles so obtained to undergo autolysis.

Parenterally introduced proteins, therefore, are apparently absorbed in large measure by the circulating blood corpuscles.

97 (1161)

## Toxicity of foreign sera for the isolated mammalian heart.

By W. H. MANWARING, ARTHUR R. MEINHARD and HELEN L. DENHART.

[*From the Department of Bacteriology and Immunity, Leland Stanford, Jr., University.*]

Seven per cent. to 10 per cent. goat serum in Locke's solution perfused under constant pressure and temperature through the

coronary arteries of an isolated normal rabbit heart, usually produces the following series of phenomena:

1. An initial tachycardia, lasting about three minutes, succeeded by

2. A period of apparently normal heart action, lasting about five minutes, succeeded by

3. A secondary tachycardia, lasting about two minutes, ushering in

4. A period of decreasing rate and strength of heart action, increasing irregularities, etc., usually ending in inactivation of the heart in about ten minutes.

If goat serum is separated into diffusible and non-diffusible fractions by dialysis through a celloidin membrane, and the two fractions are tested independently, the following results are usually obtained:

1. The diffusible substances tested in 7 per cent. to 10 per cent. dilution usually produce an initial tachycardia indistinguishable from the tachycardia from the whole serum. This is succeeded by a period of regular rate and rhythm usually lasting for over an hour.

2. The non-diffusible substances (serum colloids) similarly tested usually give no initial tachycardia, the rate and rhythm continuing unchanged for about ten minutes. There is then usually a slight secondary tachycardia, ushering in a period of decreasing heart action, usually ending in inactivation in about fifteen minutes.

The secondary tachycardia is always accompanied by a progressively decreasing rate of perfusion through the coronary arteries, and beginning myocardial edema. We are therefore inclined to attribute the secondary tachycardia and subsequent heart-death to a breaking down of the capillary defenses (increased capillary permeability), allowing the foreign colloids to pass out of the capillaries into the tissue spaces, thus coming into direct contact with the essential myocardial cells.

98 (1162)

## Analysis of the anaphylactic and immune reactions by means of the isolated mammalian heart.

By W. H. MANWARING, ARTHUR R. MEINHARD and HELEN L. DENHART.

[*From the Department of Bacteriology and Immunity, Leland Stanford, Jr., University.*]

The heart of a rabbit sensitized to goat serum, tested in a blood-free condition by perfusion with 7 per cent. to 10 per cent. goat serum, is more resistant than a normal rabbit heart similarly tested. The increased resistance is shown by the absence of the initial tachycardia, the absence or delayed development of the secondary tachycardia, and a prolongation of the life of the isolated organ.

Hearts of rabbits sensitized or immunized by repeated injections with goat serum, are more resistant than those sensitized with a single injection.

Normal rabbit serum, corpuscles or defibrinated blood, added to the perfusion fluid, decreases its toxicity. The antitoxic action of defibrinated blood is apparently equal to the sum of the antitoxic actions of its serum and corpuscles.

Anaphylactic rabbit serum similarly added usually markedly increases the toxicity of the perfusion fluid. Such an anaphylactic serum mixture may completely inactivate a normal heart within from two to four minutes. Hearts of anaphylactic and immune rabbits are more resistant than normal hearts to such mixtures.

The active principle of the anaphylactic serum responsible for this increased toxicity is thermo-labile, the toxin-increasing or toxin-producing power being completely lost, if the serum is heated to 60° C. for 30 minutes.

The active principle is not complement, since such inactivated anaphylactic sera cannot be reactivated by the addition of unheated normal serum.

The active principle is presumably not precipitin, since the specific precipitins of rabbit serum are not destroyed, or at least not completely destroyed, by heating the serum to 60° C. for 30 minutes.

Such inactivated anaphylactic sera are strongly antitoxic. The presence of a thermo-stable antitoxin in the unheated anaphylactic serum is apparently masked by the relatively strong thermo-labile toxin-increasing or toxin-producing substance.

This thermo-stable antitoxin is present in larger amounts in the sera of rabbits sensitized or immunized by multiple injections, than in rabbits sensitized by a single injection.

Sera of partially immunized rabbits (3–5 injections) added to the perfusion fluid, usually give a non-fatal shock with normal hearts. The heart may come to a complete standstill by the end of four minutes, may remain inactive[1] for from two to four minutes, and then recover completely within two or three minutes. A heart that has passed through such a non-fatal shock will usually continue to beat strongly and regularly for an hour or more.

Sera of highly immunized rabbits (8–12 injections) added to the perfusion fluid, usually give no shock, and show only a marked antitoxic action.

99 (1163)

## Autolysis of anaphylactic and immune tissues.

By W. H. MANWARING and RUTH OPPENHEIMER.

[*From the Department of Bacteriology and Immunity, Leland Stanford, Jr., University.*]

The post-mortem autolysis of normal, anaphylactic and immune guinea pig livers was followed by determining the changes in the relative amounts of coagulable and non-coagulable nitrogen (Kjeldahl method). The anaphylactic guinea pigs had been sensitized by a single injection of egg-white or goat serum. The sensitizing dose varied from 0.1 c.c. to 2 c.c. Analyses were made from 11 to 17 days after the injection. The immunized guinea pigs had been injected at 4–7 day intervals with from 3 to 7 doses of the same antigens. They were analyzed from 8 to 12 days after the final injection. A summary of the data so obtained is shown in the following table:

[1] The coronary perfusion is made under constant pressure, and is only partially dependent upon heart action. The perfusion of the myocardium, therefore, continues during the inactive period.

| | Total N per Gram. | Percentage of Non-coagulable N. | | | | Post Mortem Autolysis. |
|---|---|---|---|---|---|---|
| | | Immediate. | 6 Hrs. | 24 Hrs. | 3 Days. | |
| Normal..... | 0.034 gr. | 10.5 | 13.5 | 20 | 23 | 12.5% |
| Anaphylactic... | 0.032 gr. | 13.5 | 16 | 21.5 | 26 | 12.5% |
| Immune..... | 0.029 gr. | 14.5 | 16.5 | 22 | 30.5 | 16 % |
| Selected cases .. | .022–.026 gr. | 16–18 | 18–20 | 25–30 | 40–45 | 26 % |

The table shows a slight decrease in the average total N per gram of liver tissue in the anaphylactic animals, and a distinct decrease in the immune animals, the decrease being particularly marked in certain selected cases.

The table also shows a distinct increase in the average percentage of non-coagulable N in both anaphylactic and immune animals, confirming data recently published by Pick and Hashimoto.[1]

Contrary to their findings, however, the anaphylactic livers showed no increase in the amount of post-mortem autolysis.

A distinct increase in post-mortem autolysis, however, was observed in the immune livers, the phenomenon being particularly marked in certain selected cases. The selected animals were for the most part guinea pigs in which a marked Arthus phenomenon had been produced.

100 (1164)

## Hepatic bacteriolysins. (Preliminary report.)

By W. H. MANWARING and HARRY C. COE.

[*From the Department of Bacteriology and Immunity, Leland Stanford, Jr., University.*]

If pneumococci are deposited by perfusion methods in the liver of a normal rabbit, in the presence of normal rabbit blood, and the infected organ is now incubated at 37° C., a slight multiplication of the deposited pneumococci takes place. After 5 or 6 hours, the tissues begin to be distinctly overgrown by the microörganisms.

[1] Pick and Hashimoto, *Arch. f. exper. Path. u. Pharm.*, 76, 1914, p. 89; *Zeit. f. Immunitätsf.*, 21, 1914, p. 237. Compare also Barger and Dale, *Biochem. Jour.*, 8, 1914, p. 670.

If pneumococci are similarly deposited in the liver of an actively immunized rabbit, in the presence of immune rabbit blood, a gradual decrease in the deposited pneumococci is observed. By the end of 5 or 6 hours' incubation, the tissues have usually become relatively sterile. The few remaining microörganisms usually multiply later to form distinct colonies. The microörganisms in the larger hepatic blood vessels, not in contact with the specific parenchyma cells, are not so destroyed.

This hepatic destruction of the pneumococci is not associated with leucocytic accumulations, nor is it necessarily accompanied by phagocytosis by the endothelial cells. There is apparently an hepatic mechanism in the immune animals for the extracellular destruction or digestion of the microörganisms. Pneumococci taken up by the endothelial cells are apparently protected to a certain extent from this destruction.

101 (1165)

## A method for the determination of small amounts of sugar in urine.

By V. C. MYERS.

[*From the Laboratory of Pathological Chemistry, New York Postgraduate Medical School and Hospital.*]

All human urines probably contain small amounts of sugar, as has quite recently been pointed out by both Cole[1] and Folin,[2] who have described tests for the detection of this small amount of sugar. It has been found possible to determine this reducing substance by precipitating the creatinine and uric acid, and probably other interfering substances with picric acid as suggested by Folin for his qualitative test, and then employing a technique similar to that introduced by Benedict and Lewis[3] for the estimation of the sugar of the blood.[4] It is presumed that the re-

[1] Cole, S. W., *Lancet*, 1913, II, 861.

[2] Folin, O., *J. Biol. Chem.*, 1915, XXII, 327.

[3] Lewis, R. C., and Benedict, S. R., *J. Biol. Chem.*, 1915, XX, 61. See also Myers, V. C., and Bailey, C. V., *J. Biol. Chem.*, 1916, XXIV, 147.

[4] In a recent conversation with Professor S. R. Benedict, he informed me that Mr. Oesterberg, of the Cornell Chemical Laboratory, had likewise utilized this method for urine.

ducing substance in question is glucose, although this has been found difficult of positive proof. This question is being further investigated.

The method is carried out as follows: About 2 grams of dry picric acid are added to 10 c.c. of urine in a test tube and the tube vigorously shaken. The tube is now stoppered and placed in an ice box at 0° C. After the tube has stood for an hour, it is again shaken and then allowed to stand over night in the ice box, after which the mixture is filtered through a small filter paper into a dry test tube. The filtrate now contains less than 0.1 mg. of creatinine per c.c., a quantity too small to invalidate the estimation of the sugar. If the urine has reacted negatively to Benedict's qualitative reagent, the filtrate is diluted 1–5 or 1–10 with saturated picric acid solution. If the qualitative test has shown a small amount of sugar, a greater dilution is made. The following rule may be followed: for 0.1 per cent. of sugar dilute 1–5, for 0.2 per cent. dilute 1–10, for 0.3 per cent. dilute 1–15, etc. To develop the color, pipette 3 c.c. of the diluted fluid into a tall, narrow tube graduated to 10, 15 and 20 c.c., add 1 c.c. of saturated sodium carbonate solution and heat in a beaker of boiling water for 15 minutes. The tube is now thoroughly cooled and diluted with water to the mark most satisfactory for colorimetric comparison. Either pure glucose in saturated picric acid or a standardized picramic acid solution may be used as standard.[1]

INFLUENCE OF THE INGESTION OF GLUCOSE ON THE SUGAR OF THE URINE

| Time[2] A. M.–P. M. | Sugar of Blood, Per Cent. | Sugar of Urine, Per Cent. | Benedict's Qualitative Reaction for Sugar in the Urine. |
|---|---|---|---|
| | 0.12 | 0.09 | |
| 9–10 | 0.15 | 0.10 | Negative |
| | 0.17 | 0.17 | |
| 10–11 | 0.23 | | |
| | 0.22 | 0.90 | Strongly positive |
| 11–12 | 0.16 | 0.41 | Positive |
| | 0.15 | 0.32 | Slight cloudiness |
| 12– 1 | 0.14 | | |
| | 0.14 | 0.27 | Slight cloudiness |
| 1– 2 | 0.13 | | |
| | 0.13 | 0.25 | Very slight cloudiness |
| 2– 3 | 0.09 | 0.17 | Negative |

[1] See Myers and Bailey, *J. Biol. Chem.*, 1916, XXIV, 150.

[2] 75 grams of glucose by mouth at 9.15.

Normal urine appears to contain between 0.08 and 0.2 per cent. sugar. Urines which give only a slight reaction with Benedict's qualitative reagent give higher figures with this method, generally between 0.25 and 0.35 per cent. The data on the previous page from a human adult, kindly loaned by Dr. Bailey,[1] nicely illustrate several of the points in question.

The above results scarcely appear to support the recent conclusions of Taylor and Hulton[2] regarding the assimilation limit of glucose. If, however, only the twenty-four hour specimen of urine had been examined as in their experiments, the result would, no doubt, have been negative.

102 (1166)

## Regeneration in the mesencephalon of Amblystoma.

By **H. SAXTON BURR.** (By invitation.)

[*From the Department of Anatomy of the Yale University School of Medicine, New Haven, Conn.*]

In April of the present year the writer published a report of an experimental study of regeneration in the forebrain of *Amblystoma*. The results showed that the removal of the cerebral hemisphere together with the end-organ normally connected with it (the nasal placode), was not followed by a regeneration of nervous tissue. On the other hand, when the cerebral hemisphere was was removed, leaving the nasal placode in place as a functionally active organ, complete regeneration of the hemisphere occurred. It was concluded that the functional activity of the nasal placode provided the requisite stimulus, at first through some hormone reaction and later through the active ingrowth of the olfactory fibers, for the regeneration of the hemisphere.

This spring the same type of experiment has been performed with the ocular complex. *Amblystoma* larvæ were subjected to two series of operations. In the first the right eye and the underlying mesencephalon was removed. In the second the right eye was turned back with a flap of skin and the underlying brain removed, the eye being then returned to its normal position.

[1] See Bailey, C. V., PROC. SOC. EXPER. BIOL. AND MED., 1916, XIII, 154.

[2] Taylor, A. E., and Hulton, F., *J. Biol. Chem.*, 1916, XXV, 173.

The results are briefly these. The removal of the eye and the brain results in the formation across the gap of the wound of a curtain of tissue in all probability derived from the ependymal lining of the neural tube. The ingrowing fibers of the optic nerve from the left eye apparently stimulate the tissue thus formed to regenerate to a considerable extent. At the same time forward growing fibers from lower centers also afford some stimulus for regeneration, as was shown in the case of the primitive pallium of the telencephalon. The tissue thus regenerated is very similar in its organization to that normally found, except that important optic areas are lacking. An analysis of the fiber tracts involved must be deferred until later.

The removal of the mesencephalon leaving the eye in its normal position results in an almost complete regeneration of the optic lobes. In one larva only a very slight defect in the right mesencephalon distinguishes it from a normal unoperated individual. The process is apparently a much faster one than it is in the case of the olfactory system for the complete regeneration has occurred at the end of some three weeks, while in the case of the cerebral hemispheres complete regeneration did not occur until the end of as many months. This is really not so strange as would seem on the face of it, because, as the writer has shown elsewhere, the optic sense becomes functionally active some time before the olfactory. The early activity of the eye would then result in an early stimulus to regeneration.

These results show, as in the former experiments, that functional activity of the end-organ normally connected with the brain affords the necessary stimulus to regeneration of the part of the brain removed.

103 (1167)

## Conduction, excitability and rhythm-forming power of the atrio-ventricular connection in the turtle.

By HENRY LAURENS.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

As in the heart of the turtle *Clemmys lutaria* and of the lizards *Lacerta viridis* and *agilis* (Laurens[1]) the right and left parts of the

[1] Laurens, *Pfluger's Archiv*, 1913, 150, p. 139.

atrio-ventricular funnel of *Malacoclemmys geographica* are the portions which are most efficient in conducting the contracting impulse from the auricles to the ventricle. When the auricles are partially separated from the ventricle by a series of cuts leaving only a narrow connection, and in consequence of which atrio-ventricular block (complete or incomplete) has been brought about, it is these parts which are later most capable of conducting the impulse from the auricles to the ventricle so that the contractions of the latter follow those of the auricle coördinatedly, or so that the incomplete block is decreased.

Stimulating the funnel of beating (in situ and excised) and still hearts (first Stannius ligature) with single shocks (quick make and break) and with interrupted currents of short duration have shown (1) that the funnel is more easily excited than the base of the ventricle, (2) that the right and left parts of the funnel are more easily excited than other parts (dorsal and ventral) and (3) that the excitability of the funnel increases as one approaches the auricle.

The stimulation of the funnel just below the level of the A-V boundary of beating hearts with interrupted currents, even when these are strong and of long duration, can only occasionally produce a "fibrillation" of the ventricle or a V-A rhythm (funnel rhythm) which lasts over after the stimulation is discontinued. This is possible, however, and curves have been obtained from excised hearts showing a duration for several seconds of a funnel rhythm following a ventricular "fibrillation." In the still heart the setting up of a funnel rhythm is more easily and frequently accomplished, and several cases have been registered showing a funnel rhythm lasting for several minutes.

104 (1168)

## The influence of the vagi and of the sympathetic nerves on the rhythm-forming power of the atrioventricular connection in the turtle.

By HENRY LAURENS and C. C. GAULT.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

The investigations here reported were undertaken to determine the action of the vagus and sympathetic nerves upon the

V-A rhythm produced by electrical stimulation of the atrio-ventricular funnel. In *Malacoclemmys geographica* the two nerves are not fused into a single trunk, but run separately in the neck just median to the carotid artery. The turtles were decerebrated, and the plastron removed, the circulation being kept intact to a large degree. The vagus was stimulated just above the thoracico-abdominal ganglion, and the sympathetic, between the median cervical and the first thoracic ganglion.

Stimulation of the vagus nerves alone gave the usual results. The effects of sympathetic stimulation were, however, not so clearly marked. The general effect was a slight augmentation of the auricular contractions. Acceleration of the heart beat was less frequently obtained, the average being from 2 to 3 beats per minute, although an acceleration of as many as 6 beats per minute was registered.

Conjoint stimulation of the vagus and the atrio-ventricular funnel just below the A-V boundary with relatively strong interrupted currents produces a V-A rhythm which lasts over, in different experiments for varying lengths of time, after the stimulation has been discontinued. In these cases stimulation of the vagus nerves with a current of sufficient strength to still the normal heart causes only a decrease in the height of the auricular contraction with no effect on the rate of beat. Stimulation of the sympathetic with strong currents stops the funnel rhythm, after which a normal atrio-ventricular beat begins.

105 (1169)

## Changes in form and position of the retinal elements of normal and transplanted eyes of Amblystoma larvæ occasioned by light and darkness.

By HENRY LAURENS and J. W. WILLIAMS.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

In order to investigate the changes occasioned by light and darkness in the retinal elements of a Urodele a series of experiments on large (37 to 45 mm.) larval and on recently metamorphosed individuals of *Amblystoma* was carried out. It was found

that the pigment of these eyes undergoes a decided forward movement when the animals are transferred from darkness to light. In darkness most of the pigment is massed near the base of the epithelial cells, and only comparatively few needles extend into the protoplasmic processes between the visual cells. In light a decidedly greater amount of pigment moves toward the external limiting membrane so that the basal layer is thinner. Measurements of the distance from the external limiting membrane to the nearest pigment needle (or from the choroid edge of the epithelial cells to the farthest pigment needle) are practically the same in light and dark eyes, so that this kind of measurement gives no indication of the extent of movement of the pigment.

The cones in the light eye are 4.2 $\mu$ shorter than those in the dark eye, the total expanded length of the cones being 25 $\mu$. The rods seem to be longer in the light eyes than in the dark, but the increase is too slight to permit of satisfactory measurement.

Optic cups were transplanted at the tail bud stage to various parts of the body, where they developed to form more or less perfect eyes. The region of the auditory vesicle seemed to offer a particularly advantageous spot for the transplant. In the transplanted eyes the movement of the pigment is fully as great as in the normal eyes. The cones also contract in the light but only to the extent of about 2.5 $\mu$.

Pigment migration and cone contraction therefore do take place in a Urodele retina and can do so independently of the central nervous system.

106 (1170)

## The alleged exhaustion of the epinephrin store in the adrenal by emotional disturbance.

By G. N. STEWART and J. M. ROGOFF.

[*From the H. K. Cushing Laboratory of Experimental Medicine of Western Reserve University.*]

1. It has been stated that a marked diminution in the store of epinephrin in the adrenal gland is associated with various kinds of emotional excitation. Thus Elliott[1] speaks of morphin-"fright" in cats causing exhaustion of a gland whose splanchnic nerve

[1] *Journal of Physiology*, 1912, 44, p. 374.

supply is intact, as compared with the other adrenal whose splanchnic supply has been previously severed. We can confirm his statement as to the difference in the content produced under the influence of morphin but we do not think that fright has anything to do with the result since it is also obtained in dogs where there are no signs of fright.

2. The signs of morphin-"fright" can all be elicited by administering morphin to a cat in which one adrenal has been removed and the splanchnic supply of the other cut and in which accordingly no demonstrable liberation of epinephrin through the splanchnics takes place. A cat in this condition behaves identically in the same way as a cat whose adrenal splanchnic supply has been cut on one side but left intact on the other. The pupils are widely dilated and there is the same characteristic restlessness and incessant movement. The content of epinephrin in the remaining adrenal of the first cat is found to be practically the same as that of the adrenal removed before the administration of morphin while the content of the adrenal with intact splanchnic supply in the second cat is definitely diminished.

3. When a cat with the splanchnic supply of one adrenal cut is frightened for many hours by a dog in which also the splanchnic supply of the adrenal has been divided on one side both animals undoubtedly experience emotions of great intensity. Nevertheless the content of epinephrin in the gland whose nerve supply is intact is not sensibly diminished as compared with the other.

4. We can confirm the statement that $\beta$-tetrahydronaphthylamine causes in cats extreme exhaustion of the epinephrin store of an adrenal whose nerve supply is intact as compared with its fellow whose nerve supply has been previously severed.[1] Elliott associates this with the emotional "alarm." We have attempted to test this interpretation by making observations on rabbits.[2]

[1] Elliott, loc. cit.

[2] Division of the nerves to one adrenal is complicated in the rabbit by the fact that the right adrenal seems to derive a portion of the nerve supply concerned in changes in the epinephrin store from the left splanchnic (Kahn, *Archiv für die gesammte Physiologie*, 1911, CXL, 209; Nishi, *Archiv für Exper. Path. u. Pharmakol.*, 1909, LXI, 401). We therefore tried to eliminate the nervous connections of the left adrenal by dividing all branches going to it from the celiac ganglion and in addition cutting any strands from the lumbar sympathetic and the sympathetic itself below the diaphragm.

We have not seen nearly as great a degree of exhaustion in this animal as in the cat. This might be interpreted as in favor of Elliott's view, since signs of "emotional" disturbance are also less marked in the rabbit, although great dilatation of the pupil, increased respiration and other symptoms are present, which, according to Mutch and Pembrey[1] "give the impression that the drug produces a state of increased psychic activity accompanied by muscular action appropriate to the emotions." It seems to us, however, more natural, considering our results with morphin and "frightening" without drugs to interpret the greater effect on the epinephrin content in the cat as due to some other action of the drug than the hypothetical emotional disturbance.

We determined the epinephrin content by the colorimetric method of Folin, Cannon and Denis, which we found to agree sufficiently well with blood pressure observations on the pithed cat.

107 (1171)

## The liberation of epinephrin from the adrenals.

By G. N. STEWART and J. M. ROGOFF.

[*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.*]

The solution of the question of the liberation of epinephrin into the adrenal veins and the estimation of the amount so liberated in the absence of artificial stimulation of the splanchnics are complicated by the fact that after withdrawal of blood pressor substances are quickly developed in it, which give the same effect as epinephrin on such objects as the vessels of a frog's legs.[2] It is therefore desirable to demonstrate the fact of its liberation and to assay its amount without the necessity of withdrawing blood. We have done this (in the cat) by means of the denervated eye reactions (of Meltzer),[3] and by the effect on the blood pressure curve.

[1] *J. of Physiology*, 1911, 43, p. 109.

[2] Cf. Trendelenburg, *Archiv f. Exper. Path. u. Pharmakol.*, 1915, 79, p. 154.

[3] Experiments on the liberation of epinephrin by stimulation of the splanchnics, in which the eye reactions were used, have been described by us elsewhere, *Journal of Pharmacology and Experimental Therapeutics*, 1916, 8, p. 205.

1. For the eye reactions all that is necessary is to clamp off temporarily a pocket of the inferior vena cava so that only adrenal vein blood enters it. A clamp is applied just above the iliac veins. The renal veins are then clamped and the segment of cava emptied of blood by gently stripping it upwards. Finally a clamp is put on the cava above the adrenal veins. Only a few seconds are occupied in the adjustment of these clamps. Small branches of the segment of cava have been previously tied. The pocket is allowed to fill with blood from the adrenals. When the clamps are removed, the eye reactions are elicited at practically the same time interval as when the splanchnics are stimulated with the vessels free.

2. After section of both splanchnics (above the diaphragm) the reactions can no longer be obtained. Section of the splanchnics has therefore greatly diminished, if not abolished, the liberation of epinephrin. This is not due to the low blood pressure caused by division of the nerves. For if only the right splanchnic is cut there is little, if any, fall of blood pressure. Nevertheless when the cava pocket is closed off as described, and in addition a clamp is put on the left adrenal vein, the right being free, no eye reaction is elicited on allowing the pocket to empty itself. When the experiment is repeated with the left adrenal vein free the reaction is obtained, although of course less strongly than with both splanchnics intact and both adrenal veins open, since only half the amount of epinephrin is discharged.

3. To demonstrate the effect of epinephrin liberated into a cava pocket upon the blood pressure of the same animal, a somewhat different procedure must be adopted, in order to avoid undue disturbance of the blood pressure curve on forming and on releasing the pocket. The lower end of the cava segment is tied permanently after previous ligation of the abdominal aorta and squeezing of blood from the legs. The renal arteries and veins are also tied. When the eye reactions are available to compare with the blood-pressure curve and manipulation of the intestines is avoided during the application of the upper clamp to the cava segment, it is not always necessary that the circulation through the intestines and liver should be interfered with. Even when the blood pressure curve is somewhat irregular the rise of pres-

sure caused by the liberated epinephrin, occurring at a definite interval after release of the pocket, can be identified by the fact that the eye reaction also commences at or about this moment. However, to further strengthen the evidence we have made experiments in which the celiac and superior mesenteric arteries are first tied off, then the renal arteries, and then the abdominal aorta just below the kidneys. As much blood as possible is got into the anterior end of the animal, and then the inferior cava is tied above the iliacs. The renal veins are then ligated, and the cava pocket now represents only a blind pouch upon the circulation, the filling of which from the adrenal veins, or the emptying of which after removal of the upper clamp produces relatively little mechanical effect upon the blood pressure. The lower end of the animal is kept raised throughout the experiment. This facilitates emptying of the pocket without manipulation.

4. In different experiments we have assayed, by the injection of known quantities of adrenalin, the amount of epinephrin liberated without artificial stimulation of the splanchnics, under our experimental conditions (narcosis with urethane alone, and with urethane supplemented with ether). For example, in one experiment we found 0.0005 mg. and in another 0.0009 mg. per kg. of animal, per minute. When the pocket is allowed to fill during stimulation of the splanchnics, with intervals of rest, the effect on release is distinctly greater than when it is allowed to fill for the same time without artificial stimulation of the nerves.

5. We have endeavored to measure the amount of blood collected in the pocket, without bringing it into contact with any foreign substance, in the following way: One of the iliac veins is tied near its distal end and the other near the cava. Both iliacs are then divided distal to the ligatures. By means of the ligature on the first iliac it is suspended vertically, while the greater part of the cava segment lies undisturbed. The iliac vein thus serves as the neck of a measuring flask, so to say, the body of which is composed of the cava segment. It is not difficult to determine the moment when the blood, entering the pocket practically without resistance, the walls of the vein being scarcely at all distended so long as the vertical portion of the pocket is empty, just reaches the proximal end of the iliac. If undue exposure of

the vein is prevented, a comparison of the flow from the adrenals in successive observations is made possible by noting the intervals of time necessary for the pocket to fill up to this point. The quantity of blood required to fill the pocket can be determined once for all in each animal. The vertical position of a portion of the pocket helps to empty it without manipulation when the clamp is removed.

6. The sensitiveness of the eye-reactions to epinephrin discharged from the adrenals, for example in response to stimulation of the splanchnics, can be increased notably by temporarily clamping off alternative arterial paths. This must be done at such an interval of time after the beginning of stimulation as is not more than sufficient to allow the epinephrin to reach the beginning of the aorta. A larger proportion of the blood containing the epinephrin is thus forced to take the path to the eye whose reactions are being studied. If, for instance, the left iris is the denervated one, clamping at the proper moment of the thoracic aorta and the innominate markedly increases the reaction. It can be further increased by tying off all accessible branches of the left carotid except those through which the eye must obtain its blood supply.

108 (1172)

## Attenuation of the living agents of cyanolophia.

By RHODA ERDMANN. (By invitation.)

[*Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*]

Paschen[1] (1911) says that leucocytes must play an important part in the process of immunization. This remark seems partly justified in the attenuation process of cyanolophia. The living agents of cyanolophia are differently affected in tissue cultures of *red* bone marrow from *white* bone marrow.

[1] Paschen, O., "Handbuch der Technik und Methodik der Immunitätsforschung," 1911.

## EXPERIMENT I, 1915.

SERUM B TAKEN 36 HOURS AFTER INOCULATION OF VIRULENT BRAIN A IN A CHICKEN, SHORTLY BEFORE ITS DEATH, WAS INOCULATED IN A TISSUE CULTURE OF RED BONE MARROW AND CHICKEN PLASMA.

| Length of Time in which Virulent Serum *B* was Cultivated in Tissue Culture at 30° C. | Record No. of Chicken. | Date of Death. | Length of Incubation. |
|---|---|---|---|
| Nov. 12–Nov. 15 | No. 1 | Nov. 18 | 48 hours |
| Nov. 12–Nov. 15 | No. 3 | Nov. 17 | 38 hours |
| Nov. 12–Nov. 18 | No. 2 | Nov. 21 | 72 hours |

This proves that the virus can be kept alive six days at a temperature of 38° C. in a tissue culture of *red* bone marrow. Chickens No. 1 and No. 3 died in 48 and 38 hours. Chicken No. 2, which had been inoculated with serum B that had been kept 6 days in the tissue culture, died twenty-four hours later than the chicken which had been inoculated with serum B that had been only *three* days in a tissue culture of red bone marrow. This proves a certain attenuation by the cultivation of virulent serum in *red* bone marrow.

The living agents, which probably cause cyanolophia, can be cultivated in red bone marrow tissue cultures even longer than six days without losing their virulence.

## EXPERIMENT XII, 1916.

SERUM V TAKEN 36 HOURS AFTER INOCULATION OF VIRULENT BRAIN U IN A CHICKEN, SHORTLY BEFORE ITS DEATH, WAS INOCULATED IN A TISSUE CULTURE OF RED BONE MARROW AND CHICKEN PLASMA.

| Length of Time in Which Virulent Serum *V* was Cultivated in Tissue Culture at 38° C. | Record No. of Chicken. | Date of Death. | Length of Incubation. |
|---|---|---|---|
| Feb. 23–March 2 | No. 17 | March 4 | 48 hours |
| Feb. 23–March 6 | No. 18 | Remained alive | |
| Feb. 23–March 11 | No. 19 | Remained alive | |

So we can keep virulent the living agents of cyanolophia outside of the chicken 6–8 days at 38° C., yet in the tissue culture of *red* bone marrow the virus dies after 12 days. This is a perfect analogue to the experiment of Marchoux,[1] who cultivated the virus of cyanolophia in a culture medium which contained red blood corpuscles. He even believed that the living agents of cyanolophia had multiplied and produced a much stronger virus than that

[1] Marchoux, "Cultures in vitro du virus de la peste aviaire," *Compt. rend. Acad. Sc.*, T. 147, p. 357, 1908.

he inoculated in his cultures. In tissue cultures of red bone marrow no multiplication of the virus was observed, but a certain attenuation, as proved by the prolongation of the incubation period.

Different results were obtained by using *white* bone marrow.

## EXPERIMENT II, 1915.

SERUM C TAKEN 36 HOURS AFTER INOCULATION OF VIRULENT BRAIN B IN A CHICKEN, SHORTLY BEFORE ITS DEATH, WAS INOCULATED IN A TISSUE CULTURE OF WHITE BONE MARROW AND CHICKEN PLASMA.

| Length of Time in Which Virulent Serum *B* was Cultivated in Tissue Culture at 38° C. | Record No. of Chicken. | Date of Death. | Length of Incubation. |
|---|---|---|---|
| Nov. 30–Dec. 4 | No. 3*a* | Living | |
| Nov. 30–Dec. 6 | No. 4 | Living | |

SERUM C, THE SAME AS USED IN EXPERIMENT BEFORE, IN RED BONE MARROW AND PLASMA.

| Length of Time in which Virulent Serum *C* was Cultivated in Tissue Culture at 30°C. | Record No. of Chicken. | Date of Death. | Length of Incubation. |
|---|---|---|---|
| Nov. 30–Dec. 6 | No. 5 | Dec. 9 | 72 hours |

This experiment proves that after 4 or 6 days in white bone marrow the virus is attenuated. After inoculation of virus in red bone marrow the animal died. A controlling experiment, in which serum C was used, which was kept 7 days on ice, showed that this serum killed the chicken after 38 hours, the usual time in which this strain of cyanolophia killed the animal. It was perfectly attenuated by the cultivation in white bone marrow, partially by cu tivation in red bone marrow, and not at all by keeping the serum on ice.

The inoculation of attenuated serum and white bone marrow protected, to a certain degree, the chicken against a new inoculation, as the following experiments prove.

## EXPERIMENT III, 1915–1916.

SERUM Y TAKEN 36 HOURS AFTER INOCULATION OF VIRULENT BRAIN H IN A CHICKEN, SHORTLY BEFORE ITS DEATH, WAS INOCULATED IN A TISSUE CULTURE OF WHITE BONE MARROW AND PLASMA.

| Length of Time in which Virulent Serum *y* was Cultivated in Tissue Culture at 38° C. | Record No. of Chicken. | Date of Death. | Length of Incubation. |
|---|---|---|---|
| Dec. 26–Dec. 29 | No. 3*a* | Living | |
| Dec. 26–Dec. 29 | No. 4 | Living | |
| Dec. 26–Dec. 29 | No. 6 | Died Jan. 1 | 72 hours |

Chickens Nos. 3*a* and 4 had been inoculated with serum B and white bone marrow before, but not chicken No. 6. The same results were attained in experiments IV, V, VI, VIII, and X. All chickens which were treated with attenuated serum did not die when inoculated with a 2d or 3d dose of attenuated serum. All chickens which were not treated succumbed to the first doses of attenuated virus when it was kept only 2 or 3 days' time in tissue culture of white bone marrow. Always controlling experiments with the same untreated serum kept on ice were started, which killed the animals in due time.

It is possible to keep virulent the living agents of cyanolophia in plasma alone at a temperature of 38° for six days and longer, but the *same* virus dies in plasma, in which living white bone marrow is kept, in six days (experiment IX). The controlling experiment with serum, which was kept on ice, was positive, so it is true that the virulence of living agents of cyanolophia will be attenuated, and later die through the activity of the leucocytes. It was possible to shorten the length of time in which virulent serum was kept in tissue cultures of white bone marrow, and still inoculate it without success when the *treated animals were used again.* Animals 3*a*, 4, 7 and 8 survived after inoculation with serum $M_1$ which had been only 2 days in tissue culture (Experiment VI). Serum $M_1$ killed chicken $M_4$ after it was kept 5 days on ice, in due time. A shortening of the attenuation period to twenty-four hours was not sufficient to weaken the serum $M_2$. Chicken 7, which again was used, died in forty-eight hours, after having veen inoculated with serum $M_2$, that had only been one day in plasma and white bone marrow.

Steinhardt and Lambert[1] cultivated the living agents of vaccinia in tissue cultures of rabbit cornea. They report a definite increase of the virus, as measured by the effects of successful reinoculations. Growth of the virus could be observed in tissue cultures of the rabbit's cornea only, while heart, kidney and liver gave no results. My experiments, previously reported, prove a rapid attenuation of the virus in *white* bone marrow tissue

[1] Steinhardt, E., and Lambert, R. A., "Studies on the Cultivation of the Virus of Vaccinia, II," *Journ. of Inf. Diseases*, 1914, Vol. 14, pp. 87–92.

culture. This is quite remarkable, because the living agent of cyanolophia is not surpassed in virulence by any other virus.

The next series of experiments will deal with the attenuation of the living agents of cyanolophia in brain and liver tissue cultures and with the importance of these and the white bone marrow tissue cultures for active immunization.

109 (1173)

**Experiments on the physiology of digestion in Blattidæ.**

By ELDON W. SANFORD. (By invitation.)

[*From Osborn Zoölogical Laboratory, Yale University.*]

The question as to whether fat is digested and absorbed in the crop of the cockroach was answered in the affirmative by Professor Petrunkevitch in 1898, but in the negative by more recent authors. My investigations, which were done under the direction of Professor Petrunkevitch, show that fat is split to soluble products and absorbed in large amount in the crop, the process being observable as gradually more and more in the crop's epithelial cells at successive intervals up to forty-eight hours, and gradually less afterward. Some cells absorb so much that they appear solid black when stained with osmic acid. Ligation of the crop from the stomach does not hinder or modify the process. Fatty acids are absorbed like fats.

At certain intervals after fat feeding much fat is found in the tracheal tubes, sometimes filling them, sometimes in a thin layer on their walls, sometimes only on the supporting spirals, and sometimes mingled with chyme. This chyme resembles that normally present in the crop lumen; it is regularly present in some of the tracheæ, and in it leucocytes are often found. The chyme is evidently a normal content. The fat enters the tubes through the tracheal end cells, after being absorbed by them from the lumen of the crop.

110 (1174)

## On the transformation of the plasma clot.

By GEORGE A. BAITSELL. (By invitation.)

[*From the Osborn Zoölogical Laboratory, Yale University, New Haven, Connecticut.*]

It has previously[1] been shown by the author that in tissue cultures and in wound healing in the frog a fibrous tissue which is apparently identical with normal connective tissue may be formed by a direct transformation of a plasma clot. In an endeavor to analyze this reaction, plasma clots made from centrifuged blood plasma have been subjected to various conditions of tension and pressure. The results obtained show that with the aid of these mechanical factors it is possible to directly transform a typical fibrin net into a fibrous tissue. Judged from its histological structure when stained with Mallory's connective tissue stain, this new fibrous tissue is apparently identical with normal connective tissue of the frog. By varying the conditions it is possible to obtain preparations which will show various stages in the transformation ranging from a typical fibrin net to a fibrous tissue made up of bundles of wavy fibers such as is characteristic of normal connective tissue.

111 (1175)

## The effect of moderately high atmospheric temperatures upon the formation of agglutinins.

By C.-E. A. WINSLOW, JAMES ALEXANDER MILLER, and W. C. NOBLE.

[*From the New York State Commission on Ventilation.*]

In an earlier communication[2] we have pointed out that previous experiments on the effect of atmospheric temperature upon the development of various immunity reactions suggest two general conclusions: (1) That very high atmospheric temperatures, over 35° C., tend to produce a condition of fever and to hasten

[1] (*a*) *Jour. Exp. Med.*, Vol. 21, 1915, pp. 455–479; (*b*) *Jour. Exp. Med.*, Vol. 23, 1916, pp. 439–456.

[2] PROC. SOC. EXP. BIOL. AND MED., 1916, Vol. XIII, p. 93.

the production of antibodies of various sorts, while (2) moderately high atmospheric temperatures (30°–35° C.), apparently tend to decrease the power of producing antibodies, presumably by a lowering of general vital resistance without the stimulus which accompanies the production of fever. We reported certain experiments of our own which were in harmony with the last conclusion, inasmuch as they showed an apparent diminution in hemolysin production in rabbits kept at an atmospheric temperature of 29°–32° C. The present report deals with similar experiments upon the effect of moderately high temperature upon the formation of agglutinins.

This particular immunity reaction has been studied in relation to temperature by several observers. Rolly and Meltzer[1] kept rabbits in an incubator at 34°–38° under which condition their body temperature rose to 40°, they lost weight and showed a decrease in hemoglobin; yet when injected with typhoid bacilli they showed a marked increase both in bactericidal and agglutinating power. On the other hand Graziani[2] studied the agglutinating power of the blood of rabbits kept at lower temperatures and found, as workers on other immunity reactions have done, that moderately high heat was harmful and not helpful. The blood of rabbits kept at 2°–4° C. would agglutinate at a dilution of 1 in 1,541; at 18°, 1 in 854; at 32°, 1 in 727. In another series the blood of rabbits kept at 32° agglutinated at a dilution of 1 in 1,250, while if the animals were occasionally relieved by cold baths the agglutinating power rose to 1 in 2,425.

Studies on the agglutinating power of the blood of human beings after hot baths are conflicting. Leube[3] reports that typhoid convalescents showed a material increase in the agglutinin content of the blood after hot baths (40° for 30 minutes); while Moon[4] could not find any such increase after Turkish baths (30 minutes in a dry room at 82° C. and 20 minutes in a steam room at 54°).

In our own experiments, which were carried out in the bacteriological laboratories of the University and Bellevue Hospital Medical College, five series of rabbits, including 14 animals in all, were

[1] *Deut. Arch. f. klin. Med.*, XCIV, 1908, p. 335.

[2] *Centr. f. Bakt. Orig.*, XLII, 1906, p. 633.

[3] *Verhandl. d. Deutschen Kongresses f. innere Med.*, XXVII, 1910, p. 218.

[4] *Jour. Infect. Dis.*, XIV, 1914, p. 56.

kept (2–4 at a time) in a large incubator (12′ x 2′ x 4′) at a temperature ranging between 29° and 32° C. A similar series of 13 control animals was kept at room temperature (18°–21°). The animals were immunized by giving them intraperitoneally successively increasing doses of a suspension of killed typhoid bacilli. The injections were given twice a week. Bleedings were taken at weekly intervals and serum was drawn off from the clot and diluted with sterile salt solution.

For microscopic agglutination tests, one loopful of diluted serum and one loopful of a twenty-four hour broth culture of the typhoid bacillus were mixed on a cover glass, and a hanging-drop mount made. This was incubated at 37° Centigrade and readings made after one hour. In every case the figures in the table represent the actual dilution of serum found effective; the larger the fraction, the weaker the agglutinating power.

The results of the experiments are given in full in Table I and the averages by series in Table II. No general average can be fairly calculated because the agglutinating power of all the rabbits in Series V was so low that in a general average these Series V figures swamp all the rest and the net result depends simply on the number of Series V results included in a given week.

TABLE I.

EFFECTIVE DILUTION OF SERUM EXPRESSED IN DECIMALS.

| Series. | Heated Animals. | | | | | | Control Animals. | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rabbit. | Week. | | | | | Rabbit. | Week. | | | | |
| | | 1 | 2 | 3 | 4 | 5 | | 1 | 2 | 3 | 4 | 5 |
| I... | 3 | .00500 | .00066 | —— | —— | —— | 101 | .02000 | .00025 | —— | —— | —— |
| | 4 | .02000 | .00100 | —— | —— | —— | 102 | .00100 | .00067 | —— | —— | —— |
| II.. | 5 | .00200 | died | | | | 15 | .00050 | .00012 | .00003 | .00005 | —— |
| | 9 | .00050 | —— | .00050 | —— | —— | 16 | .00020 | .00012 | .00010 | died | —— |
| | 6 | .00200 | .00100 | .00010 | .00010 | died | 17 | .00050 | .00010 | .00010 | .00010 | —— |
| | 7 | .00200 | .00012 | .00010 | .00010 | .00012 | 18 | .00050 | .00012 | .00010 | .00010 | —— |
| | 8 | .00500 | .00012 | .00010 | .00010 | .00012 | | | | | | |
| III. | 172 | .01000 | .00025 | .00020 | .00020 | .00050 | 170 | .01000 | .00010 | .00013 | .00017 | .00025 |
| | 173 | .02000 | .00025 | .00029 | .00033 | .00050 | 171 | .01000 | .00017 | .00020 | .00025 | .00045 |
| IV. | 76 | .00018 | .00017 | .00017 | .00010 | —— | 93 | .00500 | .00029 | .00200 | .00008 | .00010 |
| | 176 | .00050 | .00017 | .00017 | .00011 | —— | 141 | .00500 | .01000 | .05000 | died | |
| V.. | 92 | .10000 | .01000 | .01000 | .02000 | —— | 44 | .10000 | .02000 | .01000 | .01000 | —— |
| | 143 | —— | .02000 | —— | .01000 | .02000 | 54 | —— | .01000 | .01000 | .02000 | .02000 |
| | 145 | —— | —— | .02000 | .02000 | .02000 | 72 | —— | .01000 | .01000 | .00400 | —— |

TABLE II.

RESULTS AVERAGED BY SERIES. EFFECTIVE DILUTION OF SERUM EXPRESSED IN DECIMALS.

| Series. | | Week. | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| I..... | Heated..... | .01250 | .00083 | | | |
| | Control..... | .01050 | .00046 | | | |
| II..... | Heated..... | .00520 | .00078 | .00020 | .00010 | .00012 |
| | Control..... | .00380 | .00023 | .00008 | .00008 | —— |
| III..... | Heated..... | .01500 | .00025 | .00024 | .00027 | .00050 |
| | Control..... | .01000 | .00013 | .00017 | .00021 | .00035 |
| IV..... | Heated..... | .00034 | .00017 | .00017 | .00010 | —— |
| | Control..... | .00500 | .00514 | .02600 | .00008 | .00010 |
| V..... | Heated..... | .10000 | .01500 | .01500 | .01660 | .02000 |
| | Control..... | .10000 | .01400 | .01000 | .01100 | .02000 |

In general our results confirm those of Graziani and suggest that a moderately high atmospheric temperature (29°–32° C.) tends slightly to decrease the power of agglutinin formation in the rabbit. In Series IV alone this was not indicated. Here both control rabbits gave abnormal results. No. 93 showed a marked drop in agglutinating power during the third week; while No. 141 never formed any powerful agglutinins and died after the third week. With the exception of this series there are sixteen weekly averages of heated and control rabbits compared in Table II. In these sixteen cases the effective dilution for heated and control animals was on two occasions the same while in the other fourteen instances a consistently larger amount of serum was needed to produce agglutination in the case of the heated animals.

112 (1176)

## Improved methods for the quantitative determination of plasma proteins.

By GLENN E. CULLEN and DONALD D. VAN SLYKE.

[*From the Hospital of the Rockefeller Institute for Medical Research.*]

The blood is drawn into a tube containing an amount of potassium oxalate sufficient to make 0.2 or 0.3 per cent. oxalate solution, and is centrifuged twenty minutes.

*Fibrin.*—5 c.c. of plasma are run into a beaker containing 100–150 c.c. 0.8 per cent. NaCl and 2–5 c.c. of a 2.5 per cent. $CaCl_2$ solution. The $CaCl_2$ may be in amounts from 2–25 equivalents of the oxalate, but about five equivalents are best. When coagulation is complete, the fibrin is filtered, the clot washed with 0.8 per cent. NaCl, and the nitrogen determined by Kjeldahl. The above is an adaptation of Howell's method for determining the activity of thrombin.[1] The filtrate from the clot may be tested for complete precipitation by addition of a solution containing thromboplastic substances.

*Albumin and Globulin are calculated from the following three determinations:*

*Total nitrogen* is determined on a 1–2 c.c. sample.

*Non-protein nitrogen* is determined in the filtrate obtained after precipitating the plasma with nine volumes of trichloracetic acid.

*Nitrogen of Globulin Filtrate:*—Globulin and fibrin are precipitated by adding to 5 c.c. of plasma 20 c.c. of $H_2O$ and 25 c.c. of saturated ammonium sulfate solution. 20 c.c. of the filtrate are mixed in a Kjeldahl flask with 3 gm. MgO "Merck's Reagent" and 350 c.c. of 50 per cent. alcohol. The solution is distilled until the distillate gives a negative test to red litmus. This takes about one hour and reduces the volume to about 20 c.c. The nitrogen, representing albumin plus non-protein nitrogen, is then determined by Kjeldahl, using 25 c.c. $H_2SO_4$. When the digestion mass becomes light brown, the sides of the flask are washed down with a few c.c. of water and ten more c.c. $H_2SO_4$ added.

*Calculation:*

Filtrate N—Non-protein N = Albumin N.
Total N—(Filtrate N + Filbrin N) = Globulin N.

[1] *Am. Jour. Physiol.*, 1910, XXVI, 453.

113 (1177)

## The response of single cells to electrical stimulation.

By **R. A. SPAETH.** (By invitation.)

[*From the Osborn Zoölogical Laboratory, Yale University, New Haven.*]

There is an accumulation of embryological, morphological and physiological evidence at hand showing that the melanophores of vertebrates are to be considered highly modified smooth muscles cells. By means of a simple recording device the responses of single melanophores of *Fundulus heteroclitus* to faradic and galvanic stimulation have been studied in some detail.

In faradic stimulation it appears that as regards the duration of the latent period, the quantity of current necessary to bring about a response in the cell, the increased height of the contraction curve with an increase in the strength of stimulus and the development of tetanus by properly spaced single break shocks, the contraction curves for a single melanophore show a striking resemblance to smooth muscle graphs obtained from the bladder of the cat (Stewart) and the stomach of the frog (Howell).

A constant current, which has previously been supposed to produce an expansion of the melanophores, causes a contraction when applied through non-polarizable electrodes of the $Zn\text{-}ZnSO_4$ type. An expansion of the melanophores may be produced by galvanic stimulation if platinum electrodes are used but this has been shown to be due to hydroxyl ions liberated at the cathode. Both the make and the flow of the constant current are effective contracting stimuli. With currents of moderate strength there is, at first, a rapid rise in the contraction curve due to the combined effects of make and flow but subsequently a partial falling off of the contraction giving a typical plateau. Stewart has found precisely the same conditions in the bladder of the cat. No response to the breaking of the constant current has thus far been observed in the melanophore.

The evidence obtained from these experiments with the responses of single melanophores to electrical stimulation, appears to strengthen and corroborate the writer's contention that in the

melanophore we are dealing with a modified and disguised type of smooth muscle cell.

114 (1178)

## Characteristics of the precipitation reaction.

By RICHARD WEIL.

[*From the Department of Experimental Medicine, Cornell Medical College, New York City.*]

In a previous communication I showed that when a chemically pure protein, such as crystallized egg albumin, is used as antigen, it combines with the precipitin of immune serum to the complete exhaustion of either factor from the mixture. From these observations the conclusion was drawn that an equilibrium subject to the laws of mass action, such as had been previously described in precipitation reactions, does not exist in these reactions, those results being attributable to the use of impure antigens, such as complex native sera. Further study has shown that chemically pure antigen unites with the precipitin in proportions that are definite and constant. The same amount of precipitinogen always "binds" an equivalent amount of precipitin, regardless of the relative excess of the latter substance in the mixture. The reverse of this statement likewise holds true. Hence it follows that it has not been possible to demonstrate the Danyz-Dungern phenomenon in the precipitation reaction when carried on with pure reagents. It appears likely, therefore, that the reaction conforms to the type of quantitative chemical reactions, and is not comparable to the adsorption phenomena exhibited by mutually precipitating colloids.

# RECAPITULATION OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE COMMUNICATIONS.

## VOLUME XIII.

1134. [with **V. C. Myers.**] Comparative distribution of urea, creatinine, creatine, uric acid, and sugar in blood and spinal fluid.

**Fitzpatrick, C. B.**

1086. The utilization of "reactor" milk in tuberculo-medicine.

**Foster, Nellis B.**

1085. See **Davis, Helen.**

1088. The isolation of a toxic substance from the blood of uremic patients.

**Fowler, C. C.**

1097. [with **Olaf Bergeim** and **P. B. Hawk.**] The availability of certain indicators in the determination of gastric acidity.

**Friedman, E. D.**

1108. [with **H. C. Jackson.**] The carbon dioxide content of blood and alveolar air in obstructed expiration.

**Gault, C. C.**

1168. See **Laurens, Henry.**

**Geyelin, H. Rawle.**

1125. Diabetes of maximum severity with marked improvement.

**Githens, T. S.**

1115. See **Meltzer, S. J.**

**Halsted, W. S.**

1065. As to the cause of the dilatation of the subclavian artery observed in certain cases of cervical rib.

**Hawk, P. B.**

1073. See **Smith, C. A.**

1097. See **Fowler, C. C.**

**Hess, Alfred F.**

1094. The influence of infantile scurvy on growth (length and weight.)

1098. An interrelationship between calcium and antithrombin in blood coagulation.

1133. A separation of serum into coagulative and non-coagulative fractions.

1144. The therapeutic effect of wheat germ and of yeast in infantile scurvy.

1143. [with **S. J. Meltzer.**] The influence of morphin upon the elimination of intravenously injected dextrose.

**Kline, B. S.**

1082. [with **S. J. Meltzer.**] Production of pneumonic lesions produced by intrabronchial insufflation of unorganized substances.

**Kolmer, J. A.**

1116. An allergic skin reaction to diphtheria bacilli.

**Kusama, Yoshio.**

1159. See **Manwaring, W. H.**

1160. See **Manwaring, W. H.**

**Lambert, R. A.**

1120. Technique of cultivating human tissues in vitro.

**Laurens, Henry.**

1167. Conduction, excitability and rhythm-forming power of the atrioventricular connection in the turtle.

1168. [with **C. C. Gault.**] The influence of the vagi and of the sympathetic nerves on the rhythm-forming power of the atrioventricular connection.

1169. [with **J. W. Williams.**] Changes in form and position of the retinal elements of normal and transplanted eyes of *Amblystoma* larvæ occasioned by light and darkness.

**Leopold, S. S.**

1066. See **Austin, J. H.**

**Loeb, Leo.**

1154. Further investigations on the cyclic changes in the mammalian ovary.

1155. See **Hesselberg, Cora.**

**Loewe, Leon.**

1106. A method for the estimation of levulose in presence of glucose.

**Longcope, W. T.**

1121. [with **F. M. Rackemann.**] Development of immune reactions in serum disease.

**Lough, W. G.**

1067. See **Myers, V. C.**

1103. See **Kleiner, I. S.**

1115. [with **T. S. Githens.**] On the augmenting action of ergotoxine (**Dale** and **Barger**) on the gastrointestinal movements.

1124. See **Auer, J.**

1131. See **Meyer, A. L.**

1143. See **Kleiner, I. S.**

**Mendel, Lafayette B.**

1146. See **Osborne, Thomas B.**

**Meyer, A. L.**

1102. [with **S. J. Meltzer.**] On continuous insufflation in fowls. A demonstration.

1131. [with **S. J. Meltzer.**] An active expiratory muscle in the chicken which is inhibited by stimulation of the central end of the vagus.

**Meyer, K. F.**

1080. See **Hurwitz, S. H.**

**Miller, H. R.**

1140. [with **Hans Zinsser.**] Complement fixation in tuberculosis.

**Miller, James Alexander.**

1073. See **Smith, C. A.**

1118. See **Winslow, C.-E. A.**

1175. See **Winslow, C.-E. A.**

**Mitchell, C. W.**

1075. See **Salant, William.**

**Morgan, T. H.**

1083. Demonstration of the appearance after castration of cock-feathering in a hen-feathered cockerel.

**Morse, Max.**

1117. See **Cutter, Irving S.**

**Mosenthal, H.**

1070. The interpretation of a positive nitrogen balance in nephritis.

**Murlin, J. R.**

1069. [with **J. A. Riche.**] The fat of the blood in relation to muscular activity and heat production.

**Rogoff, J. M.**

1170. See **Stewart, G. N.**

1171. See **Stewart, G. N.**

**Rous, Peyton,**

1107. [with **F. S. Jones.**] A method for obtaining suspensions of living cells from the fixed tissues, and for the plating out of individual cells.

**Salant, William.**

1075. [with **C. W. Mitchell.**] The action of heavy metals on the isolated intestine.

**Sanford, Elden W.**

1173. Experiments on the physiology of digestion in Blattidæ.

**Scott, G. G.**

1130. Oxygen consumption in regenerating tissue.

1145. Oxygen utilization by fishes and other aquatic animals.

**Scott, John C.**

1072. See **Ott, Isaac.**

**Simmonds, Nina.**

1136. See **McCollum, E. V.**

**Smith, C. A.**

1073. [with **Raymond J. Miller** and **P. B. Hawk.**] Studies on the relative digestibility and utilization by the human body of lard and hydrogenated vegetable oil.

**Spaeth, R. A.**

1177. The response of single cells to electrical stimulation.

**Stewart, G. N.**

1170. [with **J. M. Rogoff.**] The alleged exhaustion of the epinephrin store in the adrenal by emotional disturbance.

1171. [with **J. M. Rogoff.**] The liberation of epinephrin from the adrenals.

**Swift, Homer F.**

1122. [with **Ralph A. Kinsella.**] Immunization with sensitized bacteria.

1175. [with **James Alexander Miller** and **W. C. Noble.**] The effect of moderately high atmospheric temperature upon the formation of agglutinins.

**Woodruff, Lorande Loss.**

1153. Endomixis in diverse races of paramecium aurelia.

**Zinsser, Hans.**

1140. See **Miller, H. R.**

# EXECUTIVE PROCEEDINGS.

## Main Society.

### Sixty-ninth Meeting.

*Cornell University Medical College, October 20, 1915. President Lusk in the chair.*

*Members present:* Auer, Austin, Barber, Benedict, Draper, J. W. DuBois, Gettler, Gies, Githens, Greenwald, Halsted, Harris, Howe, Kleiner, Kober, Lee, Lusk, Mandel, J. A., Meltzer, Mosenthal, Murlin, Myers, Pepper, Pike, Riddle, Ringer, Senior, Swift, Wallace, Wiggers.

*Members elected:* B. S. Kline, H. Plotz.

### Seventieth Meeting.

*New York Post Graduate School, November 17, 1915. President Lusk in the chair.*

*Members present:* Auer, Austin, Bull, Coca, Eggleston, Ewing, James, Fine, Fitzgerald, Foster, Gies, Githens, Goldfarb, Harris, Hatcher, Howe, Jackson, Kline, Kober, Lambert, Lee, Lusk, Meltzer, Morgan, Murlin, Myers, V. C., Pepper, Pike, Ringer, Senior, Wasteneys, Weil, Wollstein.

*Members elected:* W. E. Dandy, R. G. Hoskins, E. B. Krumbhaar, Shiro Tashiro.

### Seventy-first Meeting.

*Rockefeller Institute for Medical Research, December 15, 1915. President Lusk in the chair.*

*Members present:* Auer, Benedict, Cole, R. I., Draper, J. W., Eggleston, Gies, Githens, Hartwell, Hatcher, Hess, Howe, Jackson, Kleiner, Lambert, Lee, Loeb, Lusk, Meltzer, Pappenheimer, Wallace, Weil, Wollstein.

*Member elected:* R. M. Taylor.

The Secretary read the following report from the Council:

"At a special meeting of the Council of the Society for Experimental Biology and Medicine, held Saturday, November 20,

1915, the following motion was offered, seconded and unanimously passed, seven members of the Council having been present and voting:

"On the basis of Article III, Section 2, of the Constitution, dealing with forfeiture of membership, and which specifies that 'Any member of this Society who may consent to the use of his name in any way that would aid in increasing the sale of any patent medicine, proprietary food preparation, or any similar product for which, in the opinion of the Council, inaccurate or misleading claims are made, shall forfeit his membership, *it is moved* that, in the opinion of the Council of this Society, meeting in special session, Dr. S. P. Beebe did consent to the use of his name in such a way that it did aid in increasing the sale of a patent medicine or similar product, for which inaccurate or misleading claims have been made."

It was moved by Dr. Meltzer and seconded by Dr. Auer that this report of the Council be accepted as representing the attitude of the Society in this matter. This motion was passed unanimously without discussion, twenty-two members present and voting.

**Seventy-second Meeting.**

*University and Bellevue Hospital Medical College, January 19, 1916. President Lusk in the chair.*

*Members present:* Atkinson, Auer, Benedict, Cole, R. I., DuBois, Edwards, Ewing, E. M., Fine, Gies, Githens, Greenwald, Harris, Hartwell, Jackson, Kleiner, Loeb, J., Lusk, Mandel, A. R., Mandel, J. A., Mayer, Meltzer, Murlin, Myers, Oppenheimer, Pike, Ringer, Rous, Wasteneys, Wiggers.

*Members elected:* Harold Amoss, A. A. Epstein, N. W. Janney, F. W. Peabody, Louise Pearce.

**Seventy-third Meeting (Thirteenth Annual Meeting.)**

*College of the City of New York, February 16, 1916. President Lusk in the chair.*

*Members present:* Auer, Bull, Edwards, Githens, Goldfarb, Jackson, Kleiner, Kober, Lusk, MacNeal, Meltzer, Myers, Noble, Scott, E. L., Scott, G. G., Winslow.

*Member elected:* Casimir Funk.

The meeting was held 5.00 P. M., and was followed by a dinner at 7.00 P. M. Election of officers occurred for the ensuing year after the dinner and resulted as follows:

President, Jacques Loeb; Vice-President, William J. Gies; Secretary-Treasurer, Holmes C. Jackson; Council members of the Society, J. Auer and E. F. DuBois.

**Seventy-fourth Meeting.**

*Presbyterian Hospital, March 15, 1916. President Jacques Loeb in the chair.*

*Members present:* Atkinson, Auer, Bull, Cohn, Cole, DuBois, Epstein, Fine, Githens, Greenwald, Harris, Hess, Howe, Jackson, Janney, Kirkbride, Klein, Lambert, Loeb, J., Longcope, Lusk, Meltzer, Murlin, Myers, Ottenberg, Pappenheimer, Pepper, Swift, Underhill, Wadsworth, Weil, White.

*Members elected:* Carl Ten Broeck, Edward Uhlenhuth.

**Seventy-fifth Meeting.**

*College of Physicians and Surgeons, April 19, 1916. Vice-President Gies in the chair.*

*Members present:* Auer, Berg, Eggleston, Fine, Funk, Gies, Githens, Hess, Howe, Jackson, Kleiner, Lee, Meltzer, Oppenheimer, Pike, Scott, E. L., Uhlenhuth, Zinsser.

**Seventy-sixth Meeting.**

*Yale University, New Haven, Conn., May 24, 1916. Vice-President Gies in the chair.*

*Members present:* Atkinson, Benedict, Epstein, Fine, Gies, Githens, Harrison, Harris, Hess, Hooker, Jackson, Kleiner, Kober, Lee, Lusk, Meltzer, Mendel, Myers, Scott, G. G., Uhlenhuth, Underhill, Wadsworth, Winslow, Woodruff.

*Members elected:* Walter Eddy, Rhoda Erdmann, Reynold Albrecht Spaeth, Carl Vernon Weller.

The meeting was held at 4.30 P. M., in the Osborn Zoölogical Laboratory. At the conclusion of the meeting an informal dinner was held at the Hotel Taft. Twenty-two members attended with New Haven guests.

**Pacific Coast Branch.**

**Tenth Meeting.**

*San Francisco, California, October 6, 1915.*

*Members present:* Addis, Burnett, Cooke, Dickson, Kocher, Lucas, Maxwell, Meyer, Ophüls, Walker.

**Eleventh Meeting.**

*San Francisco, California, December 1, 1915.*

*Members present:* Burnett, Cooke, Dickson, Evans, Lucas, Meyer, Ophüls, Swain, Walker, Whipple.

**Twelfth Meeting.**

*San Francisco, California, April 18, 1916.*

*Members present:* Addis, Burnett, Cooke, Crawford, Dickson, Evans, Gay, Kellogg, Kocher, Meyer, Morgan, Robertson, Whipple.

# REGISTER OF NAMES AND ADDRESSES OF THE MEMBERS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE.

ABBOTT, ALEXANDER C.....University of Pennsylvania.
ABEL, JOHN J.....Johns Hopkins University.
ADAMI, J. GEORGE.....McGill University, Montreal.
ADDIS, THOMAS.....Leland Stanford University, San Francisco.
ADLER, HERMAN M.....Harvard University.
ADLER, ISAAC.....New York Polyclinic Medical School.
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Total number of members at the close of the academic year, 1915–'16: 313.

# OFFICERS.

## 1903–1915.

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| Vice-President | Park | Ewing | Dunham | Dunham | Morgan | Morgan |
| Librarian | Lusk | Lusk | Lusk | —— | —— | —— |
| Treasurer | Calkins | Calkins | Calkins | Calkins | Calkins | Lusk |
| Secretary | Gies | Gies | Gies | Gies | Gies | Gies |

| | 1909–'10 | 1910–'11 | 1911–'12 | 1912–'13 | 1913–'14 | 1914–'15 |
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| Vice-President | Gies | Gies | Levene | Levene | Field | Gies |
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| | 1915–'16 | 1916–'17 |
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| President | Lusk | Jacques Loeb |
| Vice-President | Calkins | Gies |
| Sec'y-Treas. | Jackson | Jackson |
| Additional members of Council[1] | Gies | Auer |
| | Auer | Dubois |

[1] The Past Presidents are also members.

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## Resident (Greater New York).

*College of the City of New York.*—D. J. Edwards, A. J. Goldfarb, G. G. Scott, Thomas A. Storey.

*Columbia University.*—Russell Burton-Opitz, Gary N. Calkins, R. L. Cecil, Henry E. Crampton, George Draper, William J. Gies, P. E. Howe, R. A. Lambert, Frederic S. Lee, Isaac Levin, C. C. Lieb, W. F. Longcope, W. G. MacCallum, Thomas H. Morgan, B. S. Oppenheimer, Alwin M. Pappenheimer, F. H. Pike, E. L. Scott, Henry C. Sherman, M. J. Sittenfield, H. F. Swift. H. B. Williams, Edmund B. Wilson, Francis C. Wood, Hans Zinsser.

*Cornell University Medical College.*—Harold Bailey, Stanley R. Benedict, W. Coleman, E. F. DuBois, C. Eggleston, William J. Elser, James Ewing, Nellis B. Foster, Casimir Funk, J. A. Hartwell, Robert A. Hatcher, Graham Lusk, John R. Murlin, Charles R. Stockard, W. C. Thro, John C. Torrey, Richard Weil, C. J. Wiggers.

*Hospitals, Bellevue.*—Charles Norris. *King's County.*—B. T. Terry. *Montefiore Home.*—Nelson W. Janney, B. S. Kline. *Mt. Sinai.*—George Baehr, Charles A. Elsberg, Albert A. Epstein, R. Ottenberg. *New York.*—A. F. Coca. *Roosevelt.*—K. G. Falk, I. Greenwald, W. L. Lyle. *St. Lukes.*—A. B. Eisenbrey, L. W. Famulener.

*New York City Departments. Education.*—C. Ward Crampton. *Health.*—James P. Atkinson, Edwin J. Banzhaf, Haven Emerson, C. B. Fitzpatrick, Alfred F. Hess, Anna W. Williams, A. Zingher.

*New York Polyclinic Medical School.*—Isaac Adler.

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12 *Mt. Morris Park West, N. Y. City.*—A. I. Ringer.

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CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK CITY
OCTOBER 20, 1915

AND

TENTH MEETING
PACIFIC COAST BRANCH
SAN FRANCISCO, CALIFORNIA
OCTOBER 6, 1915

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MEDICAL SCHOOL

NEW YORK CITY

NOVEMBER 17, 1915

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NEW YORK CITY

DECEMBER 15, 1915

AND

ELEVENTH MEETING

PACIFIC COAST BRANCH

SAN FRANCISCO, CALIFORNIA

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MEDICAL COLLEGE

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JANUARY 19, 1916

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**Member elected at the seventy-first meeting:**

R. M. Taylor.

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**Dates of the next two meetings:**

January 19, 1916–February 16, 1916.

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**Dates of the next two meetings:**

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# PROCEEDINGS

OF THE

# SOCIETY FOR

# EXPERIMENTAL BIOLOGY AND MEDICINE

SEVENTY-THIRD MEETING

COLLEGE OF THE CITY OF NEW YORK

NEW YORK CITY

FEBRUARY 16, 1916

VOLUME XIII

No. 5

NEW YORK

1916

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SEVENTY-FIFTH MEETING

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NEW YORK CITY

APRIL 19, 1916

AND

TWELFTH MEETING

PACIFIC COAST BRANCH

SAN FRANCISCO, CALIFORNIA

APRIL 18, 1916

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SEVENTY-SIXTH MEETING

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NEW HAVEN

MAY 24, 1916

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*Columbia University.*—Russell Burton-Opitz, Gary N. Calkins, R. L. Cecil, Henry E. Crampton, George Draper, William J. Gies, P. E. Howe, R. A. Lambert, Frederic S. Lee, Isaac Levin, C. C. Lieb, W. F. Longcope, W. G. MacCallum, Thomas H. Morgan, B. S. Oppenheimer, Alwin M. Pappenheimer, F. H. Pike, E. L. Scott, Henry C. Sherman, M. J. Sittenfield, H. F. Swift H. B. Williams, Edmund B. Wilson, Francis C. Wood, Hans Zinsser.

*Cornell University Medical College.*—Harold Bailey, Stanley R. Benedict, W. Coleman, E. F. DuBois, C. Eggleston, William J. Elser, James Ewing, Nellis B. Foster, Casimir Funk, J. A. Hartwell, Robert A. Hatcher, Graham Lusk, John R. Murlin, Charles R. Stockard, W. C. Thro, John C. Torrey, Richard Weil, C. J. Wiggers.

*Hospitals, Bellevue.*—Charles Norris. *King's County.*—B. T. Terry. *Montefiore Home.*—Nelson W. Janney, B. S. Kline. *Mt. Sinai.*—George Baehr, Charles A. Elsberg, Albert A. Epstein, R. Ottenberg. *New York.*—A. F. Coca. *Roosevelt.*—K. G. Falk, I. Greenwald, W. L. Lyle. *St. Lukes.*—A. B. Eisenbrey, L. W. Famulener.

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*Cambridge, England.*—C. G. L. Wolf.

*Kastanienbaum, Switzerland.*—Fritz Schwyzer.

*New Brunswick, N. J.*—John F. Anderson.

*Otisville, N. Y.*—B. White.

*Princeton, N. J.*—Theobald Smith, Carl Ten Broeck.

*Rosebank, N. Y.*—Oscar Teague.

*Winnipeg, Canada.*—F. J. Birchard.

*Yonkers, N. Y.*—Isaac F. Harris.

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**Members present at the seventy-sixth meeting:**

Atkinson, Benedict, Epstein, Fine, Gies, Githens, Harrison, Harris, Hess, Hooker, Jackson, Kleiner, Kober, Lee, Lusk, Meltzer, Mendel, Myers, Scott, Uhlenhuth, Underhill, Wadsworth, Winslow, Woodruff.

**Members elected at the seventy-sixth meeting:**

Walter Eddy, Rhoda Erdmann, Reynold Albrecht Spaeth, Carl Vernon Weller.

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**Dates of the next two meetings:**

October 18, 1916–November 15, 1916

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*Cambridge, England.*—C. G. L. Wolf.
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*New Brunswick, N. J.*—John F. Anderson.
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*Princeton, N. J.*—Alfred G. Mayer, Theobald Smith.
*Rosebank, N. Y.*—Oscar Teague.
*Winnipeg, Canada.*—F. J. Birchard.
*Yonkers, N. Y.*—Isaac F. Harris.

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**Members present at the seventy-fifth meeting:**

Auer, Berg, Eggleston, Fine, Funk, Gies, Githens, Hess, Howe, Jackson, Kleiner, Lee, Meltzer, Oppenheimer, Pike, Scott, E. L., Uhlenhuth, Zinsser.

**Members present at the twelfth meeting of the Pacific Coast Branch:**

Addis, Burnett, Cooke, Crawford, Dickson, Evans, Gay, Kellogg, Kocher, Meyer, Morgan, Robertson, Whipple.

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**Dates of the next two meetings:**

May 24, 1916–October 18, 1916.

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