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Thirtieth meeting.

College of Physicians and Surgeons, Columbia University.
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1 (339)

Studies on the chemistry of anaphylaxis.

By **H. GIDEON WELLS.**

[*From the Pathological Laboratory of the University of Chicago.*]

Egg albumin, freed from the other proteins of egg white by repeated crystallization, produces typically the anaphylaxis reaction. It sensitizes in doses as small as one twenty-millionth of a gram, fatally in doses of one millionth of a gram. The minimum lethal dose for sensitized pigs is about one half a milligram by intraperitoneal injection, and about one tenth to one twentieth of a milligram when injected into the circulation. The unpurified proteins of egg white are much less active, the minimum sensitizing dose being about one hundred times greater and the minimum lethal dose being five times greater than with purified egg albumin. This suggests that inhibiting substances are possibly present in crude egg white.

The minuteness of the minimum sensitizing and intoxicating dose of pure protein seems to indicate conclusively that both the sensitizing and the intoxicating agent are one and the same kind of protein molecule, or else two different constituents of the same molecule.

Gelatin seems to be devoid of the power of participating in the anaphylaxis reaction, either with itself or with other proteins. This may be due to its poverty in aromatic radicals ; it probably is not due to the heating that is necessary for the conversion of collagen into gelatin. Addition of tyrosin to gelatin (without

chemical combination) does not modify gelatin in respect to the anaphylaxis reaction.

Milk does not lose its sensitizing or intoxicating power when heated to 100° for 30 minutes. If large enough doses of serum heated to the same degree are used they will sensitize guinea pigs to unheated serum. Coagulation with alcohol destroys or reduces greatly the toxicity of proteins which it renders insoluble in water (egg albumin) but not proteins that it does not render insoluble (serum albumin).

Pure zein is actively and specifically toxic to guinea pigs sensitized with zein, although it is devoid of tryptophane and lysine. Gliadin, which contains a less quantity of aromatic radicals than almost any other protein except gelatin, has but slight power to intoxicate pigs previously injected with gliadin.

Iodization of different specimens of serum by a constant method did not yield constant results. The partially saturated serum proteins suffered no modification in specificity analogous to that found by Obermayer and Pick in the case of the precipitin reaction. When most nearly saturated they may lose the power of sensitizing for the unaltered serum, but this is uncertain. Pure crystallized egg albumin may be saturated with iodine quite readily, the iodine saturating the unsaturated carbon atoms of the benzene ring. Such iodized albumin retains its specificity unaltered, but seems to lose much of its toxicity for sensitized guinea pigs, nor does it sensitize well to egg albumin.

Tryptic digestion of serum furnishes further evidence of the protein nature of the substances concerned in the anaphylaxis reaction. Both sensitizing and intoxicating principles are attacked, and slowly decrease in strength as the coagulable protein disappears. After 59 days' digestion of a sample of serum so that but 4.7 per cent. of the nitrogen was in a coagulable form, the sensitizing dose had been changed from one one-thousandth of a cubic centimeter to one fiftieth of a cubic centimeter, while 5 cubic centimeters intraperitoneally did not intoxicate pigs previously sensitized to bovine serum. Digestion of serum does not affect its specificity for species, but the digested serum sensitizes much better to itself than to bovine serum, and conversely.

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Further observations on the clinical aspects of hemolysis.

By **GEORGE W. CRILE.**

[*From the Laboratory of the Private Ward Service of Lakeside Hospital, Cleveland, O.*]

All cases of tuberculosis showed reverse hemolysis, *i. e.*, normal serum hemolyzed the patient's corpuscles. In all cases serum heated to 55° C. for ten minutes prevented hemolysis. Sudden chilling of the blood according to the method of Hoover and Stone caused a marked increase in the hemolytic action. Plasma obtained by immediately centrifugalizing the serum caused little or no hemolysis in hemolytic cases.

The hemolytic property reaches its maximum about 24 hours after the blood is drawn.

In the cancer group the cases in which the disease was entirely removed lost their hemolytic property in from 12 to 21 days after operation.

In cases in which an incomplete operation was performed the hemolysins continued indefinitely.

Total number of cases studied 591.

			Per cent.
Normals	211	No hemolysis	0
Pyogenic Inf.	71	7 hemolysis	10
Benign tumors	55	No hemolysis	0
Cancer	153	130 hemolysis	85
Post operative cancer cases with clinical recurrence	11	11 hemolysis	100
Post operative cases without clinical recurrence 3 weeks to 15 years after operation	37	No hemolysis	0
Tuberculosis	52	48 hemolysis	92

Our conclusion is that hemolysis occurs in a number of diseases. It occurs in great frequency in cancer and tuberculosis. The reaction in tuberculosis is the reverse of that of cancer. From the clinical standpoint hemolysis offers additional evidence which may be used in the diagnosis of cancer and tuberculosis. This evidence is not as yet specific.

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The behavior of alanin in metabolism.

By **A. I. RINGER** and **GRAHAM LUSK.**

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

Injection of 20 grams of *i*-alanin in a completely phlorhizinized dog resulted in the elimination of "extra sugar" in the urine to an amount equalling 18.8 grams, or 93 per cent. of that theoretically possible. Although *i*-alanin is almost completely convertible into dextrose, preliminary respiration experiments indicate that it does not spare fat metabolism as effectually as does dextrose itself. This may be due to heat loss in the breaking down of alanin into simpler molecules (formic aldehyde?) and heat absorption in its construction into dextrose. A similar reasoning would serve to explain Rubner's "specific dynamic action" of protein.

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An important source of error in Heller's test for urinary protein.

By **WILLIAM WEINBERGER.** (By invitation.)

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons.*]

Heller's test for urinary protein is a fairly reliable one if care is taken in its application, but several urinary protein constituents give uncertain results with it. Thus, mucin fails to yield true precipitation — the "ring" is more or less opalescent and disappears on mixing. With nucleoalbumin the ring is not quite typical and is indistinct in undiluted urine. On the other hand various misleading factors, such as resinous acids, must be taken into account. Resinous acids may be ignored, however, if such products as Balsamum Copaivæ, or Santal Oil, have not been administered, or if the specific HCl test for resinous acids shows their absence. The turbidity formed with resinous acids dissolves on warming. The acids themselves may be removed by extraction with ether. In concentrated urine, as is well known, a uric acid ring may appear just above the line of junction of the urine and acid, and urea

may be precipitated in the form of glittering nitrate crystals; but dilution of the urine prevents these effects.

In the course of our investigation of another problem, an additional source of error has been found in urines preserved with thymol in any of the usual ways. If Heller's test is applied to such urine after filtration, a ring will invariably appear even in the absence of protein. The ring is most marked, all other things being equal, in urines that have been treated with a *solution* of thymol, although it is very conspicuous in urines that have been preserved with powdered thymol.

The characteristics of this ring may be briefly stated as follows: A few seconds after the urine has been carefully poured upon the acid, there forms, precisely on the line of junction, a grayish white ring about 0.5 mm. high, resembling the albumin ring given by a faint trace of albumin, and gradually becoming more and more distinct, until, in some urines under conditions to be mentioned later, it presents the appearance of a heavy thick precipitate, the height of which increases continuously and renders the lower portion of the urine completely opaque. At this stage the color is somewhat different from that of a protein ring, in that it is more yellowish. Below the ring there is a greenish zone extending somewhat into the acid; above it, a reddish zone smaller than the former and more contracted. The white ring is seen best in daylight reflected from a dark background, the color rings are seen best if the test-tube is held against a white surface.

On slightly disturbing the layers of urine and acid the ring, if a delicate one, disappears but reappears immediately. These effects can be obtained a few times in the same mixture. A *heavy* ring, however, will not completely disappear on slightly shaking but will gradually widen and extend into the upper urinary layer; and it depends on the volume-relation between urine and acid whether complete mixing will remove the precipitate. On thoroughly shaking an excess of acid with little urine, a clear yellow solution results. If an excess of urine has been used, the mixture will not clear, but will remain turbid. Warming the mixture will not prevent the formation of this ring, nor will it clear the liquid, but, if anything, will make the reaction more distinct; nor has dilution of the urine with three to four times its volume of water any marked effect.

This reaction is caused by the fact that thymol dissolves in urine when the latter is treated with it even in solid form, and it is noteworthy that more thymol is dissolved if the urine is neutral or alkaline, than when acid. Accordingly, an alkaline thymolized urine will give Heller's test more pronouncedly than a strongly acid one. But while the thick and heavy ring in the first case will have an appearance somewhat different from the protein ring and thus will hardly mislead one, the delicate thymol ring in the acid urine closely resembles a protein ring and is therefore more apt to cause confusion.

In urine containing both albumin and thymol in various amounts each ring may be discerned. The albumin ring is somewhat the wider of the two (about 2–3 mm.), and is whitish; whereas the thymol ring forms directly underneath and is grayer and thinner. The albumin ring may be completely covered by the thick thymol ring, so that the detection of protein may be seriously interfered with.

Further investigation proved that this ring is first formed by the precipitation of thymol by the concentrated acid. At this stage it closely resembles the albumin ring. Nitration of the thymol soon occurs, resulting in the formation of nitroso- and possibly nitro-thymol. This accounts for the gradual color-change from white to yellowish white, which the precipitate undergoes. A partially successful attempt has been made to isolate the nitro-substance or substances produced. With the aid of chloroform as the solvent, crystals of a yellowish brown color were obtained that gave Liebermann's nitroso reaction. These crystals melt at a temperature slightly above 50° C., whereas nitroso-thymol melts at 160° C. Thymol melts at 50°. It is quite probable, therefore, that very incomplete nitration occurred and that the brown crystalline product referred to was a mixture of thymol and a small amount of nitroso-thymol. Besides, some chloroform may have been occluded in the crystals. Work in this direction is proceeding.

To guard against this source of error, Heller's test cannot be directly applied to urine preserved with thymol. The latter must first be removed by extraction. Petroleum ether is very suitable for this purpose. Gentle agitation of the urine with an equal volume of petroleum ether in a test-tube for 2 minutes suffices to remove all traces of thymol.

When 5 grams of thymol were administered *per os* to a medium sized dog, the urine excreted during the succeeding 24 hours showed the familiar brownish yellow color. On standing it gradually became black. Heller's test was positive but, as the resultant precipitate had the same color as the urine, the exact significance of the result was uncertain. Thereupon different metabolic derivatives of thymol — thymo-sulfuric, thymo-hydrochinon sulfuric and thymo-glucuronic acids were isolated from the urine in the form of their chlorin substitution products. A small quantity of each of these substances was then individually added to *normal* urine, which in turn was subjected to Heller's test and invariably showed a positive reaction.

Agitation of such treated urine with petroleum ether in the manner above indicated did *not* extract thymol-glycuronic acid. This fact is of some importance; for while petroleum ether readily extracts thymol from urine to which thymol has been added as a preservative, it does not quantitatively extract from urine thymol that has been given internally and which is excreted in combined forms through the kidneys.

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A clamp for direct transfusion of blood.

A demonstration.

By ISAAC LEVIN.

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

The clamp is similar in its construction to an artery forceps without the grooves. At the tip of each blade there is attached a small cannula with a smooth bore. At the inner edge of each cannula four small pin points are attached, and on the outer surface of the cannula four grooves are cut. When the clamp is closed, the pins of one cannula lie in the grooves of the other. The pins are bent outward and therefore the cannulas have a pyramidal form, so that each pin can lie snugly in its groove. At the beginning of the operation, both halves of the clamp are separated. The vein is pushed through one cannula and its wall is hooked on the pins. The same is done with the artery and the other half of the

clamp. Then both halves of the clamp are united and clamped. I believe that when we deal with small blood vessels it is easier to hook the walls on the pins than to turn them back like a cuff as is done in Crile's cannula. When the clamp is closed, both blood vessels are connected with the endothelial surfaces.

I have performed several operations on dogs, uniting the femoral or cervical vein of one dog to the femoral artery of another. The transfusion was kept up for over half an hour, until the donor was practically exsanguinated. There was no clotting, leakage, or any other defect in the clamp.

6 (344)

The further separation of antitoxin from its associated proteins in horse serum.

By **EDWIN J. BANZHAF.**

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

The literature concerning means of purification of anti-bodies and their chemical characteristics has been thoroughly reviewed by Gibson,[1] Ledingham,[2] Banzhaf and Gibson,[3] and Brieger and Kraus.[4]

Stark[5] was the first to report that by heating for one hour at 56° C., ovalbumin could be converted into a body, which, because of its precipitation and solution reactions, and also its composition, was obviously a globulin. Later Noll[6] showed the same to be true of albumin in rabbit, dog and horse serum.

My experiments were to ascertain the resulting conditions after heating antitoxic horse serum, citrated plasma, and Gibson's concentrated and partially purified antitoxic globulin solution.

An antitoxic serum by the Gibson method[1] gave the following: An elimination of 23 per cent. protein and an increase of antitoxic units per gram protein of 30 per cent. over the native serum. A

[1] *Journal of Biolog. Chem.*, I, p. 161, 1906.
[2] *Journal of Hyg.*, vii, p. 65, 1907.
[3] *Journal of Biolog. Chem.*, iii, p. 253, 1907.
[4] *Berl. klin. Woch.*, xliv, p. 946, 1907.
[5] *Zeitschr. f. Biol.*, xl, p. 494, 1900 (new series, vol. 22).
[6] *Hofmeister's Beiträge*, iv, p. 563, 1904.

series of the same antitoxic serum was heated for from 6 to 72 hours in closed containers, at a temperature of 57° C. After cooling to room temperature, the series was saturated with sodium chloride and brought up to a dilution of 1 : 10 with saturated sodium chloride solution. Twelve hours later the resulting precipitations were filtered off. Potency tests on these filtrates showed a loss of 5 per cent. after heating 6 hours and an increasing loss up to 22 per cent. after heating 72 hours. The protein converted into an insoluble condition (in saturated sodium chloride solution) was 30 per cent. for the 6-hour period, increasing up to 48 per cent. for the 72-hour period. The increase of antitoxic units, per gram protein, was 35 per cent. after 6 hours heating, increasing up to 53 per cent. after 48 hours.

Owing to the larger per cent. destruction of antitoxin at the 72-hour heating than the per cent. increase on conversion, the potency per gram protein dropped to 52 per cent. increase over the native serum. On separating the remaining unconverted albumin from this series, the increase of antitoxic units, per gram protein, was 60 per cent. after 6 hours heating, increasing to 78 per cent. after 48 hours. The 72-hour heating showed an increase of 73 per cent. over the native serum.

Citrated plasma under the same conditions gave practically the same results. Gibson's antitoxic globulin solution (blood alkalinity) containing only that globulin soluble in saturated sodium chloride solution was heated under the same conditions. The potency loss was 5 per cent. for the 6-hour period, and an increasing loss up to 23 per cent. for the 72-hour period. The soluble globulin converted into an insoluble condition (in saturated sodium chloride solution) was 30 per cent. after 6 hours' heating, increasing to 47 per cent. after 72 hours. The increase of antitoxic units, per gram of protein, was 37 per cent. after 6 hours' heating, increasing to 54 per cent. for the 24 hours. Here again the 72-hour heating period caused a larger per cent. destruction of antitoxin than per cent. globulin converted into an insoluble condition (in saturated sodium chloride solution), dropping to an increase of 46 per cent., per gram protein, over Gibson's antitoxin globulin solution. This work which is being carried out further is practically and scientifically important, and may throw some light on the chemical characteristics and the nature of antitoxins.

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Multiple tumors in mice.

By **J. W. JOBLING.**

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

During about two years we have obtained twenty-six mice with spontaneously developed tumors. Of the twenty-six mice, five showed two or more tumors of different types.

In two, the superficial and larger tumor of the two was situated on the chest wall; they were spindle cell sarcoma.

In one, in addition to the sarcoma, both ovaries were much enlarged by papillary cyst-adenomata. The other mouse with the sarcoma showed a 3 mm. wedge-shaped mass in the left lung which was not a metastasis, but a papillary cyst-adenoma.

In three other mice, the large superficial tumors were adenocarcinoma and the primary lung tumors, cyst-adenomata. It might be supposed that the lung tumors were metastases, but a study of sections showed great differences between the superficial and lung growths. Metatases show, as a rule, many mitotic figures, while the tumor of the lung, regarded as primary, show karyokinesis exceptionally. Next, the type of cell in the matastases corresponds with that of the primary tumor, besides which the cells are usually packed so closely that the cell outlines are lost, while other differences in protoplasm and nuclei occur. Again, there is little stroma in metastases, unless acini are present, in which case they are easily distinguished, while in the primary papillary growths there is a definite supporting framework. And finally, metastases tend to be invasive, while primary growths do not. The last statement is based on the point noticed that in every instance the primary growth projected from the surface and there was atelectasis of the surrounding tissue, while in metastasic nodules the growth can usually be seen extending into the alveoli without compressing the lung, or else it is confined to blood vessels. These primary lung tumors correspond very closely with those described by Tyzzer.

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On plastein.

By **D. D. VAN SLYKE** and **P. A. LEVENE.**

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

"Plastein" is the name usually applied to the protein-like substance or substances precipitated from concentrated albumose solutions by the action of enzymes. Of those who have investigated the nature of plasteins, some have agreed that they are resynthesized proteins, resulting from the reversibility of the hydrolytic reaction; others view them merely as albumoses separating from the concentrated solutions because of a lack of proper conditions to maintain solubility; while still others regard them as the insoluble simple products of further digestion of the albumoses.

Because, probably, of the small yields in which plasteins are obtained, no investigator has hitherto performed a systematic estimation of the hydrolytic products, although such estimations furnish at present our most significant data concerning the nature of proteins. We have hydrolyzed 130 g. of plastein obtained by the action of pepsin upon Witte peptone. For comparison we tabulate with our results those obtained by Brunner in Fischer's laboratory from fibrin, the mother protein of the plastein.

	From 100 g. plastein	From 100 g. fibrin (Brunner)
Tyrosine	3.03	3.1
Glycocoll	0.50	2.2
Alanine	?	3.1
Valine } Leucine }	15.59	Present 13.0
Phenylalanine	1.20	1.2
Glutaminic acid	10.02	6.8
Aspartic acid	2.15	1.7
Proline	2.55	2.4
Histidine	0.43	Not determined
Arginine	2.06	"
Lysine	1.42	"
Tryptophane	Present	Present
Total determined	38.95	33.5

Of the thirteen amino-acids tested for in plastein the presence was proved of all except alanine, which was not isolated in pure

condition. The proportions otherwise were not greatly different from those found in fibrin. It is evident that the plastein ranks with either the complex native proteins or their higher decomposition products.

In order to obtain evidence indicating with which of the above classes the plastein is to be ranked, viscosity measurements were employed. It has been shown that digestion of a protein solution is accompanied by a rapid decrease in viscosity. Consequently it appears that, under similar conditions, even its primary decomposition products form markedly less viscous solutions than the mother protein.

On comparison of equally concentrated solutions of plastein and fibrin in normal NaOH (400 mg. of dry substance to 10 c.c. solvent), it was found that the plastein solution showed much the lower viscosity. The fibrin was gradually hydrolyzed by the alkali, however, and the viscosity of its solution fell finally even below that of the plastein, which had changed but little.

For further comparison, viscosity measurements were performed upon similar solutions of hetero-albumose, and of the proteins casein, gliadin, glutein and edestine. The hetero-albumose gave a solution of viscosity similar to that of the plastein, while the native proteins all showed, like fibrin, markedly higher viscosities, and also less stability in the presence of alkali.

These results indicate that the plastein is related to the higher albumoses, and apparently, from its resistance to alkali, to the anti-albumoses rather than to the native proteins.

For the determinations, the proteins were dissolved by shaking on a machine at room temperature. All solutions were clear except that of glutein, which was cloudy. The viscosity of each was determined as soon as solution became complete, and the determination repeated at intervals. The time intervals were not regular, but figures for roughly comparable intervals are tabulated on the same line. The figures under "Hrs." indicate the time in hours between the mixing of substance and alkali, and the viscosity determination opposite. The viscosity figures indicate the rate of flow of the solutions through an Ostwald viscosimeter at $23° \pm 0.1°$, the rate of flow of water at the same temperature being taken as 100.

VISCOSITY OF SOLUTIONS OF PLASTEIN AND HETERO-ALBUMOSE.

Plastein		Hetero-albumose	
Hrs.	Viscosity	Hrs.	Viscosity
½	160.7	½	166.7
2½	156.5	1¾	161.6
8	156.0	8	154.8
20	156.3	18	150.7
——	——	30	146.9
——	——	42	146.8
52	156.1		

VISCOSITY OF VARIOUS PROTEIN SOLUTIONS.

Fibrin		Casein		Gliadin		Glutein		Edestin	
Hrs.	Viscosity	Hrs.	Viscosity	Hrs.	Viscosity	Hrs.	Viscosity	Hrs.	Viscosity
——	——	¼	266.8	——	——	—	——	——	——
——	——	——	——	——	——	—	——	2	175.9
4	223.1	5	178.0	——	——	—	——	3	169.1
8½	179.2	——	——	8½	181.4	9	247.8	10½	153.0
23	158.3	18	163.3	24	165.1	26	226.1	28	146.3
54	151.3	52	154.6	47	155.5	—	——	53	143.1

The viscosity determinations were made at the Laboratory of the U. S. Fish Commission, Woods Holl, Mass.

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The action of bile and some of its constituents upon intestinal peristalsis and the circulation.

By **ISAAC OTT** and **JOHN C. SCOTT.**

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

In their experiments with a Vella fistula, Fubini and Luzzati found that a pea, fastened to a thread, passed along the bowel more quickly when ten to fifteen minutes previously they had injected two grams of bile. C. Eckhard,[1] of Giessen, also studied the influence of the bile upon the peristaltic movement of the small intestine.

Eckhard experimented upon rabbits. He used a sodium chloride bath and studied the movements of the intestine *in situ* after opening the abdomen. After the injection into the duodenum of one cubic centimeter of bile of the rabbit the duodenum remained for ten minutes in absolute rest. He injected three c.c. of bile of rabbit, calf and sheep in different parts of the small intestine with the

[1] Eckhard: *Centralblatt für Physiologie*, 1889, p. 49.

same result, the intestine remaining quiet fifteen to twenty minutes. If, however, the intestine remained for some time in the salt solution, it became more sensitive to the irritant. With the injection of large quantities of bile there was more frequently than before little wave-like movements, although Eckhard does not feel sure that this was the result of the bile injected.

Drs. Hallion and Netter[1] have studied the influence of bile on the peristalsis of the intestine. They operated on dogs curarized or narcotized by chloralose or by morphine and chloral. By a small button hole in the small (mainly in the duodenum) intestine they introduced a balloon which was connected with a water manometer by means of rubber tubing. The balloon was flexible rubber and mounted upon a metal tube perforated by a large number of lateral openings, which prevented bends of the balloon upon itself. The water manometer was connected with a Marey tambour which inscribed the movements. After the balloon was inserted the abdomen was closed by a suture and the movements registered for more than an hour, so that subsequent curves produced by the bile could be closely compared. The bile was injected either into the blood or into the intestine. Ox bile was used, concentrated by desiccation at a low temperature, but subsequently when used diluted to its original volume by water. Then 10 c.c. of bile was injected into the rectum. At the end of four minutes repeated movements of defecation ensued, followed by an irregular rhythm for eight minutes. The intravenous injection of bile (3–7 c.c.) was by the saphenous vein. There was produced a marked diminution of peristalsis and a relaxation of tonus in the smell intestine. Immediately afterwards the contractions and the tonus considerably increased. Bile put in contact with the intestinal mucous membrane exercises a local excito-motor effect upon the small intestine. Intravenously it produces the same effect, a result that is due, in part at least, to an augmented secretion of bile by the liver, induced by the cholagogue influences of the injected bile.

Dr. Albert Schüpbach,[2] working in the Hallerianum under Professor Asher, has studied the effect of bile on the movements of

[1] Hallion and Netter: *Comptes Rendus de Biologie*, 1907, pp. 182 and 254.

[2] Schüpbach: *Zeitschrift für Biologie*, xxx, pp. 1–41.

the small intestine. Dr. Schüpbach made experiments with a Vella fistula in two dogs; also upon the rabbit's large intestine in situ, and by the Magnus method with the isolated intestine of the cat and also upon the rectum in dogs.

In the dogs with Vella fistula he made experiments with a ball of sealing wax, with a thread attached to a little weight. The bile was injected into the intestine. He also found that psychic irritations by holding ham near the nose of the animal excited increased peristalsis. When the bile was injected he noted how fast the ball moved in the intestine. The bile was mixed with physiological salt solution, or with milk, or with water. He also used the Magnus method of excised intestine.

He concludes that bile in the dog either has no special influence upon the small intestine in normal conditions or in many of the cases has an inhibitory effect. In the case of the implantation of the gall bladder into a Vella fistula, the gall of the dog had no special effect upon the peristalsis of the small intestine. In a state of hunger and at different hours after taking nourishment, the action of the bile was indifferent or a weak inhibition of peristalsis ensued.

In rabbits under ether and morphine, with the small intestine *in situ*, the bile acted in an inhibitory manner. The excised cat's intestine was inhibited by bile. The large intestine of the rabbit *in situ* had its peristalsis increased by bile. When through an injection of gall the large intestine had increased peristalsis, the small intestine remained quiet. Bile injected into the rectum of the dog called out defecation.

Our experiments were made upon etherized rabbits and cats. They were thirty-eight in number. We used two methods in the study of intestinal peristalsis. The first one was that of Magnus on the excised intestine in a modified Ringer solution with oxygen bubbling through it. The other was the insertion of a rubber balloon into about the middle of the jejunum in the small intestine and in the ascending colon of the large intestine. This was connected with the delicate piston recorder of Dr. Schlayer, of Tübingen. The bile used was that of cats and of rabbits.

Effect of bile on small intestine. — With the method of Magnus small and large doses locally applied decreased peristalsis. In one

case, a cat, there was with one eighth of a drop an increase of tonus and a slight augmentation of peristalsis. In the balloon method, bile given per jugular or injected into the lumen of the intestinal part experimented upon decreased peristalsis. In the rabbit, with one eighth of a drop by the Magnus method a temporary, well marked inhibition of peristalsis was seen with a decrease of extent of peristalsis. In a cat by the balloon method, one half a drop per jugular increased peristalsis. In a rabbit, one quarter of a drop by the jugular produced an increased peristalsis with the balloon method.

Effect of bile on the large intestine. — The effect of bile on the large intestine by the Magnus method was to decrease tonus and peristalsis of the intestine. In rabbits there was occasionally, after a $\frac{1}{16}$ to a $\frac{1}{32}$ of a drop of bile by the Magnus method, greatly increased peristalsis. In the balloon method, bile in the cat per jugular in doses of a drop, decreased tonus and peristalsis at first, and afterwards greatly increased them.

Effect of glycocholic acid upon the small intestine. — In the cat, by the balloon method, three quarters of a grain of glycocholic acid per jugular increased the tonus and the extent of the peristaltic movements. In other cats, one half grain of glycocholic acid decreased peristalsis, using the balloon method and injecting the acid per jugular.

In the rabbit, $\frac{1}{128}$ of a grain of glycocholic acid by the Magnus method greatly decreased the tonus and peristalsis.

Effect of glycocholic acid upon the large intestine. — In the rabbit, glycocholic acid by the balloon method in one half grain doses per jugular decreased peristalsis. In a rabbit and a cat it increased peristalsis producing quite large waves.

Effect of taurocholic acid on the small intestine. — In the cat, one half grain of taurocholic acid greatly increased peristalsis by the Magnus method. In the rabbit there was a momentary increase of tonus by the Magnus method.

In the rabbit, with $\frac{1}{16}$ to $\frac{1}{2}$ grain of taurocholic acid there was with the Magnus method a great decrease of tonus and peristalsis.

In the balloon method, $\frac{1}{4}$ of a grain of taurocholic acid per jugular in the cat increased peristalsis after a temporary decrease.

EXPERIMENT 1. *Cat.*

Bile on blood pressure.

Time P. M.	Bile.	Rate 15 sec.	Height, mm. Hg.
2.00.00		45	158
2.01	¼ gtt.	45	156
2.02		43	160
2.08		43	156
2.10		45	150
2.10.30	¼ gtt.	46	154
2.12		46	156
2.18		48	160
2.20		47	160
2.20.10	½ gtt.	49	158
2.22		50	160
2.27		50, clot.	158
2.35		56	144
2.35.30	1 gtt.	54	142
2.37		57	150
2.43		64	142
2.45		56	140
2.45.10	2 gtt.	54	128
2.47		55	138
2.53		57	128
2.55		64	130
2.55.10	2 gtt.	68	110
2.57		56	124
3.03		56	124
3.05		55	122
3.05.10	4 gtt.	58	80
3.07		58	104

EXPERIMENT 2. *Cat.*

Taurocholic acid on blood pressure.

Time P. M.	Taurocholic acid.	Rate 15 sec.	Height, mm. Hg.
2.00.00		45	132
2.00.15	¼ grain	43	130
2.02		42	132
2.05		44	132
2.10		44	134
2.11		43	134
2.11.20	¼ grain	45	124
2.12		43	132
2.15		40	130
2.20		42	130
2.21		40	132
2.21.20	½ grain	44	112
2.22		43	116
2.25		43	130
2.30		41	130
2.31		40	126
2.31.10	½ grain	47	104
2.32		45	120
2.35		40	124
2.40		40	122
2.41		40	124
2.41.30	½ grain	43	100
2.42		42	118
2.45		42	124
2.50		39	120
2.52		35	116
2.57		35	114
3.02		35	113
3.07		35	114

EXPERIMENT 3. *Rabbit.*

Glycocholic acid on blood pressure.

Time P. M.	Glycocholic acid.	Rate 15 sec.	Height, mm. Hg.
3.00		45	118
3.00.40	⅛ grain		
3.01		42	115
3.02		46	115
3.06		46	110
3.08		47	110
3.08.10	¼ grain		
3.08.30		45	108
3.09		46	110
3.14		42	108
3.16		42	108
3.16.30	¼ grain		
3.17		42	106
3.21		43	110
3.23		43	114
3.23.30	½ grain	44	90
3.24		42	110
3.26		30	96
3.28		47	104
3.30		44	108
3.30.15	½ grain		
3.31		45	96
3.35		31	84
3.37		34	104
3.37.30			
3.38		48	64
3.38.30		40	80

Effect of taurocholic acid on the large intestine.—By the Magnus method, $\frac{1}{2}$ grain of taurocholic acid decreased peristalsis and the tonus of the large intestine. In the rabbit, $\frac{1}{4}$ to $\frac{1}{2}$ grain of taurocholic acid by the Magnus method greatly decreased the tonus and peristalsis.

There is no doubt that either with the excised intestine or with the balloon method, where the nerves of the intestine are attached, that bile from $\frac{1}{32}$ of a drop up to 4 c.c. primarily inhibits peritalsis and afterwards may or may not increase peristalsis. The taurocholic and glycocholic acids have the same action as the bile itself. The experiments upon the pulse-rate and arterial tension show that bile after a few doses reduces the tension and the heart beat. Taurocholic acid reduces the heart beat and the blood pressure. Glycocholic acid reduces the blood pressure, but did not materially alter the pulse-rate.

From an examination of our results it is apparent that bile has contradictory effects upon the small and large intestine. It is evident that with either the Magnus method or the balloon method results are antagonistic. Dr. Schüpbach believes that this may in part be explained by a psychic reaction on the intestine in his experiments. It will not, however, explain the contradictory effect in the case of the excised intestine. In 1884, in a paper on intestinal peristalsis, Ott called the ganglia in the intestine intestino-motor and intestino-inhibitory. It is probable that these ganglia have a varying antagonistic effect, and as one or the other is in the ascendant we have an increase or a decrease of peristalsis by the bile. It is evident that the circulatory changes did not have any part in the changes in intestinal persitalsis.

10 (348)

The uric acid excretion of normal men.

By **PAUL J. HANZLIK** and **P. B. HAWK.**

[*From the Laboratory of Physiological Chemistry of the Department of Animal Husbandry of the University of Illinois.*]

The purpose of the investigation was to observe the course of the excretion of uric acid in normal men living on an ordinary mixed diet. Each subject was allowed to select his own diet and

then was required to ingest the diet selected during the course of six periods of four days each. Ten university students served as subjects. Quarters were provided where the men could easily be observed as to certain regulations of sleep and diet. The body weights of the subjects ranged from 53.1 kg. to 76.7 kg. and their ages varied from 19 to 29 years. There were no athletes among the subjects so that no individual took excessive or violent exercise, but all lived the life of the average, normal university student. The Folin-Shaffer method for the determination of uric acid was employed.

CONCLUSIONS.

1. The average daily excretion of uric acid for ten men ranging in age from 19 to 29 years, and fed a normal mixed diet, was 0.597 gram, a value somewhat lower than the generally accepted average of 0.7 gram for such a period.

2. The average daily protein ingestion for these same subjects, when permitted to select their diet, was 91.2 grams or 1.33 gram per kilogram of body weight.

11 (349)

Hemolysins in the sera of carcinoma and syphilis.

By **S. PESKIND.** (By invitation.)

[*From Dr. Peskind's Private Laboratory, Cleveland, Ohio.*]

A few years ago the writer commenced a research, the object of which was to determine in what diseases hemolysins commonly occurred and whether or not they were specific for these diseases. The ultimate purpose was to obtain data that one could use in diagnosis.

A preliminary report was published.[1] The work had to be abandoned shortly thereafter. Since then, other investigators — notably Kelling — have taken up the study of hemolysins in connection with their use in diagnosis.

It occurred to the writer that it would be very desirable to determine the question as to the specific nature of the hemolysins found in various diseases. With this object in view, a study of

[1] Peskind: *American Medicine*, 1903, v, p. 918.

the serum in several diseases has been undertaken. In this brief report are given the results obtained in carcinoma and syphilis.

The sera and corpuscles of 12 cases of carcinoma and 7 cases of tertiary syphilis were examined. In 10 other cases of tertiary syphilis, the corpuscles alone were examined.

The experiments were planned in groups. In each group, the corpuscles of at least one normal person, and several cases of syphilis and carcinoma were exposed to the action of sera derived from syphilitic and carcinomatous patients.

The customary technic was employed, special care being taken to use only fresh specimens of blood. Equal parts of serum and of a one per cent. saline suspension of corpuscles (washed four times) were incubated at 37° C. for one or two hours, sedimented on ice over night, and compared with control tubes of the serum.

In all 290 serum-corpuscle combinations were made.

In this way were studied the actions of syphilitic and carcinomatous sera on their own corpuscles, on normal corpuscles, and on the corpuscles of other cases of syphilis and carcinoma.

The following results were obtained:

SUMMARY OF RESULTS.

Out of the 12 cases of carcinoma, 4 showed the presence of hemolysins in their sera, which caused laking of the erythrocytes derived from normal human individuals.

Out of the 7 cases of tertiary syphilis whose sera were examined, 6 showed the presence of hemolysins which dissolved the corpuscles of normal persons.

The corpuscles showed the following behavior:

The corpuscles belonging to a hemolytic carcinomatous blood were found to be immune to the action of the hemolysins in its own serum or any other carcinomatous serum.

The corpuscles belonging to a non-hemolytic carcinomatous blood were readily laked by a hemolytic carcinomatous serum.

Similarly the corpuscles of a hemolytic luetic blood were found to be immune to the action of its own serum or any other syphilitic serum.

The corpuscles of a syphilitic blood, whose serum did not contain hemolysins were laked by any hemolytic syphilitic serum.

In every instance, it was found that the corpuscles belonging to a hemolytic carcinomatous blood were immune to the action of the hemolysins found in syphilitic serum. Conversely, the corpuscles present in a hemolytic syphilitic blood were immune to the action of the hemolysin present in carcinomatous serum.

However, the corpuscles of a non-hemolytic carcinomatous blood were readily laked by hemolytic syphilitic serum and similarily the corpuscles of a non-hemolytic syphilitic blood were laked by a hemolytic carcinomatous serum.

Judging from the behavior of the sera towards the corpuscles derived from various normal and diseased persons, one could not distinguish a hemolytic syphilitic serum from a hemolytic carcinomatous serum.

This would suggest that the hemolysin found in syphilitic serum is identical with one found in carcinomatous serum.

It would seem, from the above results, that there is some connection between the presence of a hemolysin in the blood and the immunity of the corpuscles contained in that blood.

The corpuscles found in hemolytic bloods — whether from cases of carcinoma or syphilis — are immune to the action of the hemolysins in those sera. The corpuscles of non-hemolytic bloods are vulnerable and are readily laked by the hemolysins contained in either carcinomatous or syphilitic sera.

The reverse proposition could be argued from the results of the above experiments. That is, if the corpuscles of a given blood are immune to the action of a hemolytic serum, then the blood in question contains a hemolysin. If the corpuscles are laked by a hemolytic serum, then the blood in question does not contain hemolysin.

The corpuscles of 10 other cases of tertiary lues (whose serum was not obtained) were tested against the hemolysins in carcinomatous and syphilitic sera.

The corpuscles of 3 of these cases were easily laked by hemolytic sera derived from carcinomatous or syphilitic persons.

The corpuscles of the other 7 cases were found to be immune to the action of the hemolysins.

It is reasonable to suppose that the 3 bloods with the vulnerable corpuscles did not contain hemolysins, while the 7 bloods containing immune or resisting corpuscles did contain hemolysins.

It was found that the addition of 30 per cent. of normal serum was sufficient to inhibit the action of the hemolysins in both the syphilitic and carcinomatous sera.

Normal serum seems to possess protective substances which inhibit the action of the hemolysins. Hemolytic sera also may possibly contain the same protective substances as normal sera. These substances, if present, would neutralize a certain amount of the hemolysin existing in the serum.

If only a small amount of hemolysin be present, it would, if the above conditions actually exist, be neutralized and rendered incapable of detection by the technique used at present. The corpuscles belonging to such a serum, however, would still be immune to hemolytic carcinomatous and syphilitic serum.

The serum of one case of tertiary lues was found to contain so little hemolysin as to be barely demonstrable. The corpuscles of this blood, however, were perfectly immune to either carcinomatous or syphilitic sera.

The results of this research have some bearing on the subject of transfusion. Inasmuch as some cases of carcinoma possess hemolytic sera, we could not transfuse such cases with normal blood. If transfusion of such a carcinoma case be found necessary — as a preliminary to operation or for other reasons — it will be necessary to secure as donor a person whose blood corpuscles are immune to the serum of the carcinoma case and vice versa.

According to our observations, the desired blood could be found in cases of tertiary lues that have just recovered from their lesions.

The hemolysins found in syphilis and carcinoma appear to be true isolysins. In experimental isolysin-formation the isolysin is a reaction product of the organism in which it is formed. The corpuscles of the animal in whom the isolysin is produced are immune to that isolysin. No anti-hemolysins are present in the sera of such animals.

The evident immunity of the corpuscles in the hemolytic syphilitic and carcinomatous bloods towards the hemolysins in their sera indicates that the hemolysin is a true isolysin, and, like the experimental isolysins, is probably a reaction product of the organism in which it occurs. Some toxic substances absorbed from the dis-

eased focus could be assumed as the exciting cause of such an isolysin formation.

In conclusion, the writer wishes to acknowledge his indebtedness to Dr. J. E. Tuckerman, who collaborated in the research.

12 (350)

The effect of instilling adrenalin chloride into the mammalian eye.

By **W. H. SCHULTZ.** (By invitation.)

[*From the Division of Pharmacology, Hygienic Laboratory, Washington, D. C.*]

Certain writers have concluded that mydriasis cannot be produced by instilling adrenalin into the eye of higher animals except under pathological conditions such as lesions of the pancreas or the removal of the superior cervical ganglion. Perhaps this conclusion results from an oversight of the antagonism existing between the influence of instilled adrenalin and light stimuli when simultaneously acting upon the intact eye. At any rate the conclusion is not supported by more recent experiments and is misleading when used as a basis for diagnosing certain pathological conditions.

I have found that mydriasis can be produced in these animals with relative ease and certainty. In making a comparative study, however, of different degrees of susceptibility to adrenalin, due care must be taken to keep the intensity of light stimuli constant. This is essential, since in the eyes of higher mammals where the light reflex is well developed, strong light may cause the pupil to constrict to such an extent that any antagonism of this process by adrenalin may be lost sight of. For instance, by instilling adrenalin into the normal cat eye for some minutes and then examining the eye in light bright enough to constrict the untreated eye to a small slit-like aperture, no difference in the drugged and undrugged eyes can be detected; but the same eyes examined in a dark corner may show a distinct difference in the pupils, the drugged pupil dilating more than the normal one. Thus the early dilating effect of adrenalin can be detected more easily by reducing the intensity of the light stimuli. In this preliminary com-

munication, however, it is not so much the time of initial dilation as it is the time required for complete antagonism of the light stimuli by adrenalin that is considered. The degree of resistance to this antagonism is perhaps best illustrated by the appended protocols, each taken from a series of experiments. Here it is shown that maximal dilation and loss of light reflex in the guinea pig results within 23 minutes after instilling 6 drops of 1 : 1000 solution of adrenalin chloride. After instilling 2 drops every 2 minutes into the eyes of the following, maximal dilation and loss of light reflex results : In the rabbit, within 56 minutes ; the dog, 96 ; the cat 101 ; whereas in the monkey it requires 148 minutes ; and in normal man even longer.

From the protocols it will be seen that adrenalin not only dilates the pupil of the mammalian eye, but that in each case the stimulating effect of light of moderate intensity can be completely overcome by it. Furthermore the ease with which this antagonism is accomplished seems to depend upon the degree of development attained by the light reflex mechanism, since in animals that have a sensitive light accommodating mechanism most highly developed the period of instillation and amount of solution must be increased to produce mydriasis, whereas in animals with a reflex mechanism less sensitive to light stimuli, mydriasis is relatively easy. In conclusion it seems that these facts must be considered in formulating a theory dealing with the dilator mechanism of the eye or with the value of the adrenalin test for certain pathological conditions.

1. Normal guinea pig. Sept. 5. Park, Davis & Co.'s 1 : 10000 adrenalin chloride.

10.30 A. M. Before instillation, diameter of right and left pupil about 5 mm.
10.32 " two drops instilled in right eye.
10.36 " right pupil 5.8 mm., left pupil 5 mm.
10.39 " " " 6 mm., left pupil 5 mm.
10.43 " two drops instilled into right eye.
10.45 " right pupil 7 mm., left pupil 5 mm.
10.50 " " " 7.8 mm., left pupil 5 mm.
10.51 " two drops instilled into right eye.
10.55 " right pupil 9 mm., left pupil 5 mm.
11.11 " " " 9 mm., practically dilated to a maximum.
12.32 P. M. " " about same as at 11.11.
1.15 " " " 7½ mm., left about 5 mm.
1.24 " " " about same as left.

Maximum dilation brought on by six drops. Time required was about 23 min., at the end of which time the pupil no longer reacted to light.

2. Normal gray rabbit. Sept. 3. Two drops of P., D. & Co.'s adrenalin chloride instilled every 2 minutes.

3.40 P. M. Right pupil 6.6 x 9 mm., left 6.6 x 9.0 mm.
4.02 " " " 11.0 x 12 mm.
4.12 " " " 12.0 x 12.5 mm.
4.38 " " " 13.5 x 14 mm. Last instillation: left 7 x 10.4 mm., pupil scarcely reacted to light.

3. Large adult female cat. Aug. 20. P., D. & Co. 1 : 1000. Instilled every 2 minutes.

10.30 A. M. Right and left pupils alike; transverse diameter about 1½ mm.
10.34 " first instillation.
11.48 " slight dilation of right eye, 2½ mm., left 1½ mm.
12.45 P. M. maximum dilation 11.2 mm., left 1½ mm., practically no reaction to light after 101 minutes.

4. Young adult female dog. Aug. 21. P., D. & Co.'s 1 : 1000 sol. of adrenalin chloride; two drops every two minutes.

10.00 A. M. Right and left pupils of same diameter — about 4.2 mm.
10.04 " first instillation.
10.40 " right pupil about 5 mm., left 4.2 mm.
11.00 " " " " 6.8 mm., " 4.2 "
11.16 " " " " 9 mm., " 4.2 "
11.40 " " " " 11 mm., " 4.2 " Last instillation.
4.30 P. M. " " " 2 mm., " 5 "

Maximum dilation was reached after about 96 minutes at which time the pupil ceased to react to fairly bright light from a skylight. By 4.30 the right pupil had not only ceased to dilate, but even in such a light as one might secure in a well-lighted room at 4.30 P. M., the drugged eye constricted much more than did the normal one.

5. Monkey. P., D. & Co.'s adrenalin chloride, 1 : 1000. Two drops every two minutes.

10.30 A. M. Both pupils of the same diameter, about 2.4 mm.
10.32 " first instillation.
10.46 " right pupil 2.6 mm., left 2.4 mm.
10.49 " " " 3.0 mm., " 2.4 "
11.10 " " " 3.5 mm., " 2.4 "
11.12 " " " 4.0 mm., " 2.4 "
11.35 " " " 5.4 mm., " 3.1 "
11.46 " " " 6.0 mm., " 3.0 " pupils turned toward bright light.
12.06 P. M. " " 6.5 mm., " 2.8 " constricted to about 1 mm.
12.20 " " " 7.0 mm., " 2.8 "
12.40 " " " 8.4 mm., " 3.2 " When turned toward bright light, the left pupil constricted to less than 1 mm. in diameter while the right constricted but little (8.2 mm.).
1.00 P. M. Last instillation, practically maximum dilation.
5.00 " In dim light, right and left pupils about the same.

Maximum dilation was reached after 148 minutes at the end of which time the pupil no longer responded to bright light. Greater constriction of the right eye than the left was noticed the following morning.

In normal men, the resistance is still greater than in the monkey.

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Successful canine infection with cultures of Leishmania infantum (Ch. Nicolle).

By **F. G. NOVY.**

[*From the Hygienic Laboratory, University of Michigan, Ann Arbor, Mich.*]

By the collective term *Leishmaniasis* we may designate three apparently distinct diseases (1) Kala-azar or tropical splenomegaly of India and the East; (2) Oriental sore, otherwise known as Delhi, Biskra, Aleppo, etc., boil; and (3) infantile splenic anemia. These are characterized by the presence of peculiar intracellular parasites commonly known as the Leishman-Donovan bodies. The work of Rogers and others has shown that the parasite of Kala-azar develops in a citrate solution, into flagellate or trypanosome-like organisms, but attempts at cultivation on blood agar have given negative results. The recent investigations of Ch. Nicolle on the parasites of Oriental sore and of infantile splenic anemia establish the important fact that the Leishman bodies found in these two diseases can be cultivated on blood agar with the same ease as in the case of many trypanosomes. Nicolle has further shown that the infantile splenic anemia can be transmitted to dogs and monkeys by injection of suspensions of the diseased tissues, but attempts to produce an infection by inoculation of the cultures of the flagellate failed.

Having received through the courtesy of M. Mesnil, of the Pasteur Institute, transplants of the eighth generation of Nicolle's flagellate, it was decided, first of all, to test in a severe way the question as to the possibility of inducing an experimental infection in animals by means of such cultures. Accordingly a dog was given, in the interval from April 13 to Sept. 21, fifteen intraperitoneal injections of fresh vigorous cultures. The organism was grown on blood-agar at 20°, and for each inoculation the growth from a large number of tubes (8–40) was taken up in citrate solution and injected. A total of 270 cultures were thus utilized in the course of five months. The dog apparently showed no effect, other than occasional leucocytosis, and microscopic examination of the peripheral blood gave negative results.

The dog was bled and when autopsied on October 9 presented evidence of a prolonged chronic infection. The spleen was small and tough and weighed but 33 grams, the dog weighing 11.7 kilos. The liver and kidney likewise were found to be unusually hard. Microscopical examination of the spleen, liver, kidneys, lungs, and bone-marrow showed enormous numbers of typical Leishman-Donovan bodies, free and intracellular, with no sign of the flagellate form. Cultures made from the spleen and liver gave at the end of five days exceedingly rich growths of the flagellated organism.

Tubes inoculated with the peripheral blood likewise gave good cultures though somewhat later, that is, on the tenth day. The latter fact indicates the value of the cultural method as a diagnostic means.

It will be seen therefore that, starting out with the flagellate form, it has been possible to produce a typical infection in the dog and to recover from the infected animal, by cultural means, the parasite in the flagellated stage. Undoubtedly this result can also be obtained by employing less massive doses than was deemed necessary in this preliminary experiment.

14 (352)

New apparatus designed especially to facilitate the preservation of food for use in metabolism experiments.[1]

A demonstration.

By **WILLIAM J. GIES.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons.*]

The writer exhibited a new form of apparatus that has been very serviceable in the preservation of fresh food by refrigeration. The apparatus consists in the main of a galvanized "angle iron" frame constructed to support glass trays specially designed as food containers. Fresh food, *e. g.*, hashed meat, may be very satisfactorily preserved, without change of general composition, by

[1] This method further improves the process described by the author some years ago in the *American Journal of Physiology* (1901, v, p. 235). See also Gies and collaborators: *Biochemical Researches*, 1903, i, p. 69 (Reprint No. 1).

placing it in *covered* trays of the kind referred to and transferring them immediately to such a frame in a freezing room. The general characters and relationships of the main parts of the apparatus are clearly shown in Figure 1. The structure and dimensions of the glass trays are indicated in detail in Figures 2 and 3. See pages 30 and 31.

In the form of this apparatus now in use in this laboratory, each "angle iron" skeleton is 20 inches high, 21 inches wide and 10 inches deep. Twenty five glass trays fit snugly into as many stalls,[1] which are just a trifle wider and longer than the trays, and are arranged in five tiers. The removable horizontal rods at the front are so arranged as to prevent the trays from falling from the frame if the latter happens to be tilted forward. The "angle iron" fixtures at the rear prevent movement of the trays in that direction when the frame is tilted backward.

The glass trays are the essential parts of the apparatus and are excellent food containers. Plates of ordinary glass furnish very satisfactory covers for the contents of the trays. Such a glass lid, trimmed to fit intimately, can easily be put in place and can readily be elevated with a finger at the depression in the edge of the tray, at one end. Air tight closure may be secured by placing over the tray a full-width strip of paraffined muslin before the closely fitting lid is pressed down tight upon the ledge in the tray which supports it.[2] Paraffined muslin will not appreciably absorb moisture from the contents, nor freeze fast to the latter, and can be washed free from the slight amounts of food that may adhere to it. If such a paraffined muslin strip is allowed to extend a little beyond one end of the tray, the protruding portion serves as a means of drawing the tray from the frame and also of lifting the lid of the tray. Such paraffined muslin strips may be used again and again.

The trays are composed of thick flint-glass and therefore are able to withstand unusally rough treatment. Neither the lids nor the trays have been cracked by alternate cooling and warming between the extremes of temperature to which they have com-

[1] Five additional trays may conveniently be placed directly underneath the lower tier of trays in the frame.

[2] Such closed trays full of meat may be kept weeks at a time in a refrigeration room without losing weight.

monly been subjected, *i. e.*, 5° C. and room temperature, nor have the lower temperatures made the glass brittle.

The covered trays hold about 550 grams of hashed, expressed, lean meat, or 325 grams of cracker meal.[1] The freezing of a closed trayful of lean meat is devoid of any appreciable expansive effects.

This method of food preservation, where the necessary refrigeration facilities are at hand, offers the following special advantages:

The trays are in effect bottles that rest on one side and open on the opposite side. The paraffined muslin cover and the glass lid may be removed together as easily as a stopper may be taken from a bottle. The trays can be filled or emptied easily and quickly. The thick side of the tray furnishes a very stout fulcrum for strong leverage with a heavy knife through frozen food, such as hashed meat. Consequently, frozen food in such a tray may easily be sectioned with a knife into blocks without any risk of breaking the tray.

The trays are comparatively shallow. Therefore, percolation of liquid in fresh food (such as the juice in hashed meat) before freezing sets in must be very slight, if it occurs at all.[2] The influence of such possible percolation on the uniformity of composition of portions removed daily is negligible, especially if the food is used in sections *cut from top to bottom.*

The uniformity of the dimensions of the tray makes it easy to mark off very accurately given quantities of any relatively homogeneous product. Upright partitions, of paraffined card-board for example, may be used between weighed quantities of food, placed side by side, without any danger of admixture or difficulty of removal.

Since the trays can easily be marked for identification, many different dietary mixtures can be systematically preserved at the same time in the apparatus described, and may be used separately without confusion. After fresh food has been frozen, trays containing it may safely be kept in an ordinary refrigerator for a day or more, thus increasing the convenience of handling material preserved in this way.

[1] The covered trays hold about 575 c.c. of water.

[2] Thus far no visible percolation has occurred, in such trays, in meat previously expressed in an ordinary "tincture press."

FIG. 1. A view of the "angle iron" frame holding seven glass trays. Two additional trays in different positions, with their glass lids leaning against them, are shown. The white objects in the foreground are paraffined muslin strips, which are used with the glass lids to effect air tight closure of the trays.

Tray I in the second stall of the first tier has a glass lid in place, which shows distinctly in the picture. The trays in the stalls diagonally downward to the right of Tray I, and also the tray in the second stall of the lowest tier, are empty and without lids. The two covered trays in the fourth and fifth stalls of the second tier contain frozen hashed meat and show the usual appearance of full trays in reserve in the frame.

Trays II and III outside the frame show clearly the inside appearance of the trays.

The removable horizontal rods are in position to prevent forward movement of the trays. The special turns on the ends of the rods should be noted.

It is needless to dwell upon the obvious fact that the apparatus described may be used very satisfactorily for many other purposes. The trays are excellent crystallization dishes, for example, and serial recrystallizations may be effected in them very conveniently.

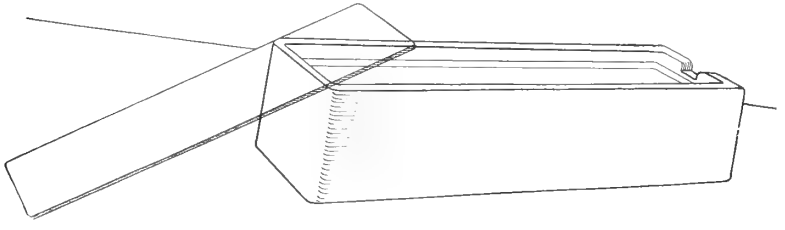

FIG. 2. A diagram of a tray with the lid out of place, to show especially the ledge upon which the lid rests when the tray is covered. The depression in the edge of the tray at one end, where the lid may readily be lifted with a finger, is also indicated sharply. This depression does not extend to the surface of the ledge. Therefore, when the lid is in place, the tray is completely closed.

The general structure of the apparatus permits its placement in out of the way positions. The whole of it can readily be protected by an easily removable impervious covering.

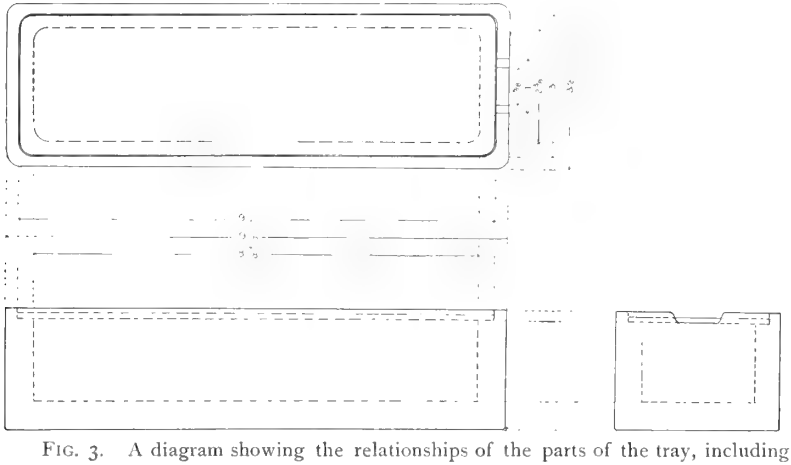

FIG. 3. A diagram showing the relationships of the parts of the tray, including the lid, and giving the main dimensions.

I am indebted to my assistants, Mr. Walter H. Eddy and Dr. Archibald E. Olpp, for the photograph and drawings of the apparatus. Mr. Christian Seifert, of this laboratory, executed admirably the plans for the frame. The glass trays were made to order by the Whitall Tatum Co.

Thirty first meeting.

Rockefeller Institute for Medical Research. December 16, 1908.
President Lee in the chair.

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Reply and explanation to recent criticism of my experimental study on effects of extirpation of the salivary glands on the gastric secretion.

By **JOHN C. HEMMETER.** (By invitation.)

[*From the Physiologic Laboratory of the University of Maryland, Baltimore.*]

It is not always a congenial task to have to reply to a criticism of one's experimental work. To many a conservative thinker, the policy contained in a remark attributed to Ludwig under a similar circumstance, "Schweigen ist gold," may appeal as more expedient. But yet, the dignified silence may be interpreted, by the one who has advanced the criticism and even by the research worker and general student of physiology, as a tacit approval to the fault finding — in other words, as signifying that the criticism was deserved and the work criticised defective. I find myself in this embarrassing position with regard to an article published in the "Proceedings of the Society for Experimental Biology and Medicine, 1908, v, pp. 114–117," New York, by Dr. A. S. Loevenhart and Dr. D. R. Hooker, entitled: "Note on the supposed presence of a gastric hormon in the salivary glands."

Although the physiology and pathology of digestion has been my life work, yet, as one of the results of many years of laboratory teaching and training, I am loathe to insist dogmatically on any of my opinions and am ready at any moment to be corrected and to advance another step in the attainment oftruth. ("Experientia fallax, Experimenta mendax.")[1]

[1] But rather than dwell upon the moral side of scientific controversy I prefer to refer to Sir Thomas Browne's "Religio medici," 1904 edition, p. 98.

Especially welcome are such corrections when they emenate from such an esteemed friend and talented worker as Dr. Loevenhart. The original worker whose results are criticised has the right, however, to demand that his special point of inquiry ("Fragestellung") and all the methods of experimentation, operative, physiologic and chemic, shall be conscientiously repeated on, at least, an equal number of the same kind of animals, successfully nursed through the identical operative procedures. He has a right to demand a scrupulous regard for detail, and for all the finer distinctions made in his application of methods, some of which may have required years for their perfection in his hands and those of his associates.

Let us investigate whether my friend, Dr. Loevenhart, has fulfilled these indispensable, fundamental conditions that should precede destructive criticism.

I sought to ascertain the effect of salivary gland extract in dogs deprived of all four pairs of salivary glands, whose gastric juice had been carefully studied before any operation of removing the glands was undertaken. Sometimes the removal had no very marked effect; but in those dogs in which it did, I tried to ascertain whether the depressed gastric secretion could be restored or not by salivary gland extract. I tried to study the effect on a secretion already abnormally depressed in three series of dogs — thoroughly recovered from the operation, allowing ten days to two weeks, at least, for recovery.

Dr. Loevenhart starts with normal dogs, as he supposes, and expects to raise the gastric secretion qualitatively and quantitatively *above* the normal. He seeks the effect of salivary gland extract in raising a supposedly normal gastric secretion to a higher acidity and proteolysis — an entirely different problem from mine.

I have never published anything on the effect of salivary gland extract on the normal gastric secretion of dogs. It is not asserted that this extract can raise the gastric secretion *above normal*, but only that it may, under certain conditions, partially restore a gastric secretion that is depressed *below* normal. Dr. Loevenhart is attempting to change a normal secretion to an abnormal (higher) one. I studied the effect in restoring an abnormal secretion to a normal one.

When there are four different procedures for obtaining gastric juice on the same dog within thirty minutes, and the jugular vein exposed, a cannula inserted and submaxillary extract injected intravenously, it must not be overlooked that, with every additional interference, the animal becomes more and more disturbed and that this seriously influences his gastric secretion. The chemico-physical and the neuro-physical processes of secretion are thoroughly upset unless a long time for recovery is given. This is shown in Dr. Loevenhart's results, page 4 of his reprint, in which the total acidity and free HCl and the proteolytic power became less and less in specimens *A*, *B* and *C*; only when the psychic secretion was aroused, granting that this was not a delayed effect of injection sal. gl. extr. specimen *D*, was there any notable proteolysis without addition of acid. The notes of the beginning of experimentation on this dog bear the date of April 6, and the qualitative studies bear the date of April 8 — not near time enough to permit dog No. 2 to entirely recover.

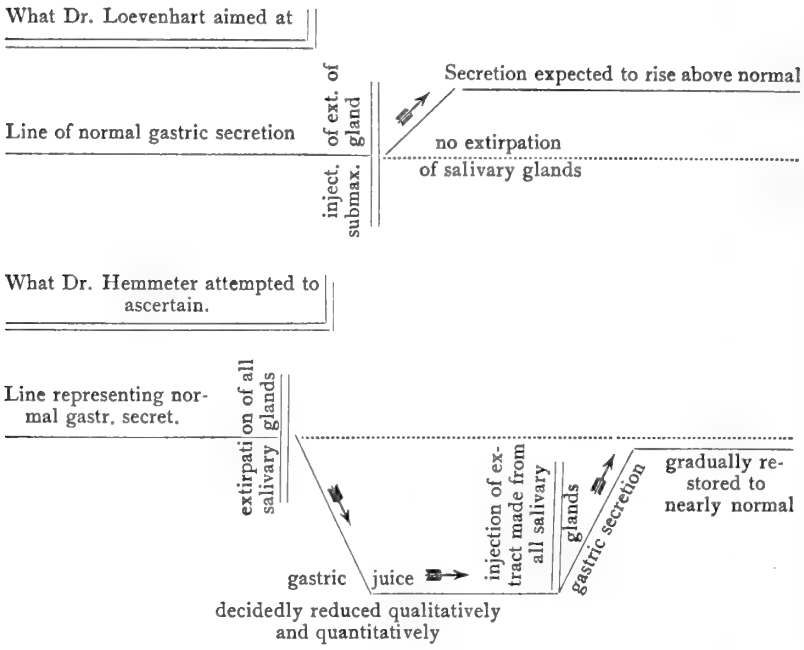
To expect salivary gland extract to raise the gastric secretion qualitatively and quantitatively above what is the regular standard for the average dog is to expect something abnormal — for an unusually abundant and unusually active gastric juice is logically as abnormal as one that is unusually diminished or inactive.

1. What Dr. Loevenhart presumes is that the salivary gland extract should change a normal gastric juice to an abnormal one (from the regular amount to an unusually high amount and activity).

2. What I attempted to ascertain was whether or not an abnormal gastric juice could be restored to the normal (from diminished and weakened secretion to the normal). The "Fragestellung" is not the same, in fact, it is highly digressing.

Dr. Loevenhart observed only two animals. Nowhere does he give the date of operations, nor state the time that elapsed between the operation and first day of experimentation, nor the amount of proteolysis in millimeters of Mett tubes. Both animals were abnormal. The first dog, No. 1, he admits had distemper and was feverish, was thin and would not eat. The first observations are dated November 11, 1907, and this animal died within 48 hours.

All of our results were gained from dogs that lived for three to six months and then had to be killed in most cases because we had no room or facilities for keeping them during the summer vacation — excepting the series of the summer of 1907 when I kept four dogs at our country home.



Dog No. 2 of Dr. Loevenhart was also abnormal. This is evident from the feeble proteolysis as indicated by Mett tubes (in Dr. Loevenhart's article they are called "Metz" tubes) and the low acidity, and Dr. Loevenhart gravely states that the fluid gained by catheterization, 8.6 c.c., specimen *A*, contained *much dark mucus* (blood? and mucus). Mucus in a fasting dog's stomach is one of the most reliable indications of gastritis. Dog No. 2 had a diseased stomach also.

We made our salivary extract from maceration of all four pairs of canine salivary glands, even the orbital — and it is all important that this extract should be made only from salivary glands that have been functionally active immediately before their excision

(the dog must be made to chew bread and then rapidly etherized). I have worked with extracts of inactive glands, but so far have refrained from publishing anything concerning their effects or non-effect. In not a single instance, has the operative plan and technique used by us, nor the physiologic routine of preparing the glands by functional work, nor the chemic discipline of ascertaining the proteolytic activity been punctiliously carried out by Doctors Loevenhart and Hooker.

Both of the animals had diseased stomachs. In neither were the salivary glands extirpated. The entire plan of experimentation and aspect of physiologic inquiry is so fundamentally different from mine, that comparison of their work with ours is not logical, and any deductions from their work as used to interpret our results are unfortunately misapplied.

It is only fair that the work of an experimentor should be judged from his most recent publication, in this case that which appeared in the *Biochemische Zeitschrift* (Hamburger Festschrift, Band xi, p. 238), the only complete report published by me.

The short notice by which Drs. Loevenhart and Hooker judged our work was nothing but a preliminary report, and contained, as such reports occasionally do, some inaccuracies which I have taken the privilege to correct in the article published in the *Biochem. Zeitschr., l. c.* ("Die Wirkung der Total Extirpation Sämtlicher Speicheldrüsen auf die Sekretorische Funktion des Magens beim Hunde"). Even in this article the printer has allowed some wrong figures to slip into the headings of tables C, D and F, pp. 257, 258 and 259, for which I am in no way responsible, but which do not injure the main argument, especially as the editors of the *Biochem. Zeitschr.* politely corrected them in a subsequent *Berichtigung*.

In Dr. Loevenhart's experiment on April 8, submaxillary extract was injected into dog No. 2 at about 3.10 to 3.15 P. M., the gastric juice of twenty minutes later showed a free HCl of 0.20 (titration with *n*/20 NaOH) but the proteolytic power with addition of acid is declared to be "*good.*" But at 3.30 the stomach of the same dog was catheterized and specimen *A* obtained after the dog was allowed to smell meat for ten minutes. This specimen *A* was the most active that Loevenhart obtained. It came 35

minutes after the submaxillary extract was injected. Question is: Would not this active juice have been secreted even without the efforts to cause a psychic secretion, for the salivary extract in my experience has a latent period in which it produces no very marked secretion? After that period it may come; that is, a pronounced secretion may come, even thirty minutes after injection of salivary extract and even if there has been no chance for psychic secretion. Pawlow, *l. c.*, p. 70, states that in all cases the latent period after the vagus stimulation of gastric secretion may be from 15 minutes to one hour, and even more.

Hitherto we have known the term "*latent period of secretion*" only in connection with the stimulation of a nerve going to gland or muscle. We are not so familiar with the use of the term "latent period" in connection with the chemical stimulation of a gland. A moment's reflection will bring the thought nearer to us that even after nerve stimulation, pure and simple, chemical events must transpire in the gland cells which require a certain time for their elaboration. Now, if the stimulation is purely chemical, and not through a nerve, the same or similar chemical events must precede the actual outpouring of secretion. We are still ignorant of the processes that occur during the "latent period," but recent work indicates that they are partially electrical and partially of a chemical nature. We must also consider that the immediate effect of a chemical stimulation, like the immediate effect of a nerve stimulation, may be inhibited.

There are so many side influences of a physical, nervous and chemical nature which control the phenomenon of the "latent period" that its exact nature and what transpires during it, is still a matter of speculation.

It may, at first sight, seem paradoxical that the latent period of secretion after sham feeding in dogs is stated by Pawlow to be only 5 to 10–15 minutes, and the latent period after vagus stimulation 15 minutes to one hour — for in both instances the stimulation is transmitted by one and the same nerve to the identical synapses in the gland cells. Pawlow explains this, p. 71, *l. c.*, by his belief that in artificial stimulation of the vagus, the stomach receives the excitatory as well as inhibitive impulses, and the latter check secretion.

How can we conceive of inhibitive processes to explain a long latent period of secretion, when chemical substances (for example, salivary gland extracts) are injected intravenously? By an analogous experimental reasoning, we have learned (Pawlow, *l. c.*) that it is impossible to imitate the influence and action which the vagus exerts during normal life while digestion is going on, for our laboratory methods are far too coarse and the complexity of fibers in this magnificent highway of nerve tracks too intricate for us to single out individually functioning secretory fibers.

We are not much better off when we attempt to imitate the chemistry of the internal secretion of glands, for only in a single instance has a hormon been isolated in a state that reveals its exact chemic structure.

The chemic messengers are bodies of definite chemic structure which are released with unerring exactness from their producing organs; but when we manufacture an organ extract, it is, of course, possible that we may seize the hormon (if I may still use the term); but unavoidably we must extract the entire tissue of the organ and as a result obtain extracts, which contain materials that stimulate, but also materials that may inhibit secretion. This occasional inhibitive effect of salivary gland extract on gastric secretion has brought to mind two ideas: either that I am not dealing with a hormon or stimulator at all, or that there may be two kinds of chemic correlation, one that stimulates and the other that inhibits. The conception which sees an antagonistic, as well as a synergistic, correlation brought about by chemic messengers is at least as rational, when applied to the physiologic correlation of organs by means of chemic substances communicated to them by means of the circulation, as when applied to the correlation of organs by means of nerve elements. This relation of organs by means of reciprocal (antagonistic or synergistic) action of nerves is not new to physiologists, and has been brought home to us in a most impressive manner by Meltzer, not to mention Ch. S. Sherrington, New York, 1907. All of this is still hypothesis; but this hypothesis has been given color (1) by the seemingly paradoxical effects of (*a*) such a pure substance as adrenalin, which does not always cause constriction of vessels (only when they are severed from the nerve centers) but sometimes may cause dilatation, when in normal animals a certain vascular area is intact in connection

with its nerve centers,[1] and of (*b*) gland extracts, which sometimes raise blood pressure and often lower it (sometimes after a slight previous rise), and (2) by the contradictory effects of some salivary gland extracts on gastric secretion. All of this doubt will continue so long as we are compelled to deal with a complex mixture of various substances in gland extracts and not with one pure substance of known composition.

To this consideration belongs, also, the antagonistic phenomena reported by Lilienfeld, Morowitz and Delezenne as occurring in blood coagulation (positive and negative phase of coagulation). This is explained by Lilienfeld and also by Delezenne by the isolation from blood plates and leucocytes of two substances, one of which they term "*leuconuclein*" which favors coagulation, and the other, "*liston*," which retards coagulation. Before the isolation of these two substances the phenomenon of the positive and negative phase during blood coagulation appeared paradoxical, and the idea of a **latent period of coagulation** might have come to many an experimentor. Just so with the latent period after chemical stimulation of the glands; it may be due to inhibitive substances in the gland extracts used, and it is possible that this delay in bringing about the effect after chemical stimulation of the gastric glands, may disappear with a clearer knowledge of the chemistry of the gland extracts, and a more accurate method of preparing them.

Besides the latent period of secretion, we must consider the neutralization of the first acid secreted by the mucus present in the stomach. Pawlow ("Arbeit. d. Verdauungsdrüsen," *l. c.*, p. 39) calls attention to what he emphasizes as "Factum," namely, "*Even with a normal stomach and with a pure gastric juice 25 per cent. of its acidity can be lost through neutralization by mucus.*" How much more must this neutralization take place in a stomach that, as Loevenhart states, gave "*much dark mucus.*" The very efforts of catheterization increases the mucus formation, and after the submaxillary gland extract was injected, if it had any stimulating effect at all (I am not prepared to state whether it had or not) this much is sure, the mucus had to be neutralized before

[1] This latter effect of adrenalin is not a purely chemical effect but a mixed effect of nerve and chemical phenomena. One and the same chemically pure substance cannot be claimed to contain both stimulating and inhibitive substances.

there could be free HCl. The extract was injected at 3.20 on April 10; at 3.25 P. M. the gastric juice was drawn by catheterization (8.8 c.c., specimen *C*). No free HCl was in it, but six minutes after the injection of salivary extract the dog was shown meat, and ten minutes after that there was a fourth catheterization (the fourth in 30 minutes). This 5.3 c.c. was active juice and Loevenhart and Hooker attribute it to psychic secretion.

Considering the latent period of secretion and the time for neutralization by mucus, it is reasonable to inquire whether or not the injection of extract had a feeble but delayed influence, although Loevenhart and Hooker used only submaxillary extract and not that of all four pairs of glands, and did not prepare it in the manner I did.

Concerning the inflammation (gastritis) in the stomachs of their dogs, I can very readily appreciate the difficulty, for I had been thwarted and misled by diseased canine stomachs for almost a year before we gradually learned to recognize, avoid and treat them.

Evidences like these, naturally suggest that such experiments cannot be successfully carried out in a few months. I was not aware of Dr. Loevenhart's criticism, until November 14, 1908. That there are salivary extracts that have no peptogenic effects whatever, and others that are variable, I have already stated in my article in the *Biochemische Zeitschr.*, Vol. xi, p. 251 ("Verschiedenheiten in d. peptogenen Kraft d. Speicheldrüsen Extrakten").

Then again, the complexity of the mechanism of gastric secretion in dogs is such (*Biochem. Zeitschr.*, *l. c.*, p. 253) that the initial depression caused by extirpation of the salivary glands probably may be gradually replaced by special efforts of the remaining sources of stimulation to the gastric glandular apparatus.

This problem is far too deep and complicated to have years of laborious experimentation set aside by a casual testing of two sick dogs, as to whether a saline extract of the inactive submaxillary gland alone can cause a secretion of gastric juice in animals not deprived of their salivary glands.

That there may be defects in my work I am willing to accept as a possibility, because a general knowledge of the history of physiology reveals the status that the first results of similar ex-

perimental work are only in most exceptional instances without defects or errors.

Such a defect in the connexus of cause and effect has recently been brought to my knowledge and, today, makes it debatable whether the name "hormon" is correctly applied by myself to the stimulating quality of one gland extract upon the secretion of another set of glands. The definition and conception of the hormon allows a rather wide application, it is true, but it seems to me it ought to be restricted to substances whose chemical structure is at least approximately known and that have one predominant characteristic or specific effect on other glands, in which effect they cannot be replaced by extracts from other organs or tissues. This is not the case with the salivary extracts, for, as we can learn (*Biochem. Zeitschr.*, Vol. xi, p. 253), extracts of the pyloric mucosa and of the spleen (Luciani) act in a similar manner in stimulating gastric secretion.

Concerning the pepsinogenous effect of the spleen on the gastric secretion, I refer to the work of Tarulli and Pascucci, executed in Luciani's laboratory and described in the latter's splendid work, "Physiologie des Menschen," translated into German by Baglioni and Winterstein, Vol. ii, pp. 151 and 152. On page 153 it will be seen that the extract must be made from an *active* spleen, as Luciani says "a spleen that is hyperemic and swollen," which means, taken from a dog during the height of the digestive period. Extracts of spleen taken during the period of functional rest had no pepsinogenous effect; but the meaning of Luciani and his pupils above mentioned is unmistakable. A chemical substance is formed in the spleen during its activity which, when brought into the circulation, is absorbed by the gastric glands and is capable of augmenting the quantity of the secreted pepsin. Additional emphasis is given in these experiments to the fact that the extract should only be made from a functionally active gland.

Whatever may be the final outcome of investigations concerning the chemical nature of the hormones, Bayliss and Starling consider that they were originally accidental by-products of the activity peculiar and proper to the organ which has produced them. Thereafter the next step in the development of a correlation is the acquisition of a sensitiveness or a responsiveness to the hormones

in any remote organ ("Die Chemische Koordination der Funktionen des Körpers," *Ergebnisse der Physiologie*, Jahrgang v, p. 670). The only word to which I could take exception in this explanation of Bayliss and Starling is the word "accidental" ("*Zufällige*" Nebenprodukte). I should like to enlarge this conception when applying it to the digestive tract, and state that the various segments of the digestive tube are correlated and coordinated by a sensitiveness not *only to accidental products*, but to the regular by-products which are known to accompany the formation of the specific products of the organs of digestion.

An infirmity in the experimental logic, suggestive of a metabolic by-product produced in the salivary glands during activity which might be regarded as a chemical messenger to the secretory apparatus of the stomach, might be found in the occasional failure to produce total loss of gastric secretion after the salivary glands are removed. In other words, we should expect to find invariable "*Ausfalls-Erscheinungen*," phenomena of lapse or total deficiency of gastric secretion. That these do not occur after the salivary glands are extirpated with that regularity that is necessary to justify the use of the term "*hormon*," is at least partially explained by the existence of several other sources wherefrom the secretory apparatus of the stomach may receive its stimulations; these other sources have been sufficiently considered in the preceding and in the *Biochemische Zeitschrift*, Vol. xi, p. 253.

I do not wish to be understood as asserting that an extract of the inactive submaxillary gland alone can have an effect in raising the amount and proteolytic activity of gastric juice, but only, that, if it possibly could exert such an effect, not sufficient time was allowed after the injection in Dr. Loevenhart's experiments to adequately test this point of inquiry.

If there is anything of importance that has revealed itself to us since the publication in the *Biochemische Zeitschrift*, Vol. xi, p. 238, it has come through experimental study of the occasional long latent period after injection of some salivary extracts and not after others. This has suggested the existence of chemic substances which inhibit or check gastric secretion. These substances, if they exist as definite chemical bodies, must be more abundant in resting, than in functionally active, salivary glands.

There is nothing contradictory in the idea that one and the same gland cell in one segment of the digestive tract may contain two kinds of chemical messengers for the succeeding segment of the digestive apparatus. One kind stimulates secretion in the following segment and a second kind inhibits or arrests it.

Starling ("Recent Advances in the Physiology of Digestion," p. 90) speaks only of *hormones* (from *ορμάω*, to excite, arouse or stimulate). But on reflection it must be evident that for the normal regulation of life processes, it may, under certain conditions, be equally important that any process of secretion or vascular tonus should be capable of inhibition by chemical messengers. Two such diagonally opposed chemical substances which are concerned in coagulation have been isolated from lymphocytes by Lilienfeld and Delezenne, one of which *leuconuclein* favors coagulation and a second *histon* which inhibits it. The leuconuclein corresponds to the *hormones* but the *histon* is an inhibitor. For such chemic bodies — physiologic arresters like *histon* — I would suggest the name *koliones* from the Greek *κωλύων*, to inhibit, to prevent, arrest or check.

16 (354)

A critical study of the conditions under which zymase and its associated co-enzyme bring about alcoholic fermentation.

By **GEORGE H. A. CLOWES.**

[*From the Agricultural Chemical Laboratory of Professor Buchner in Berlin, and the New York State Laboratory, Buffalo.*]

Zymase, the enzyme of yeast discovered in 1896 has since been proved by Harden and Young to consist of two parts, (1) zymase proper, an enzyme-like body possessed of high molecular complexity, non-diffusible and thermo-labile, and (2) a readily diffusible, thermo-stabile, relatively simple, chemical complex, which, for lack of a better term, has been designated as the co-enzyme of zymase.

Harden and Young separated the bodies in question by diffusion, but owing to the paucity of their materials and the destructive effect exerted by secondary causes during the lengthy process

involved, it was found impossible to obtain any clear insight into the conditions of physico-chemical equilibrium obtained in this reaction. Buchner's *dauerhefe*, that is to say, a preparation of pressed yeast precipitated by an excess of acetone or alcohol ether, can be prepared in large quantities and exhibits a high degree of resistance to the action of destructive enzymes. We therefore directed our attention to the preparation of *dauerhefe* containing as large a zymase content and as small a co-enzyme content as possible, our object being to study the effect exerted by a preparation of this nature upon fermentable sugars, when used in conjunction with varying proportions of a boiled yeast extract containing co-enzyme. It was found possible to produce a preparation of acetone *dauerhefe* which in itself alone possessed no fermentative activity whatsoever, but which when used in conjunction with a suitable quantity of boiled yeast extract, exhibited an unusually active fermentation, 2 grams mixed with 6 grams of sugar and 20 c.c. of extract producing from 1 to 2 grams of CO_2 in the course of 8 to 10 days.

Having thus demonstrated that it is possible to obtain a stable preparation containing relatively large quantities of zymase, and also to prepare a relatively stable boiled extract of yeast containing co-enzyme, a series of experiments was commenced, the object of which was to determine the effect of varying proportions of co-enzyme used in conjunction with a constant amount of zymase, and vice versa. In several series of experiments in which a constant amount of zymase (2 grams *dauerhefe*), was used in conjunction with 6 grams of sugar and from 1 to 50 units of co-enzyme, it was found that the velocities of reaction and the fermentation end results were directly proportional to the number of units of co-enzyme employed up to an optimum concentration, after which a fall in the value of both these quantities was to be observed. The same phenomenon exhibited itself when varying proportions of *dauerhefe* were employed with a constant amount of co-enzyme, other conditions being constant.

The velocity of reaction k, is calculated from the formula

$$K = ln \frac{A}{A - X} \cdot \frac{1}{T},$$

where A represents the relative molecular concentration of the sugar and $A - X$ the concentration at any given time T. The progress of the reaction is readily followed by estimating the loss in weight of fermentation tubes due to the evolution of CO_2. In all cases in which no disturbing influence has been allowed to exert an effect, this velocity of reaction is found to be constant for a period of three or four days immediately following the establishment of active fermentation. Provided all experiments are carried out at a constant temperature and that other conditions are maintained on a uniform basis, the value of K is found to be directly proportional to the product of the concentrations of the zymase and its co-enzyme, according to the formula

$$\frac{K}{K_1} = \frac{ZC}{Z_1C_1},$$

where K and K_1 represent velocities of reaction and Z and Z_1 concentrations of zymase and C and C_1 concentrations of co-enzyme in comparative series. The accuracy of this formula over a comparatively wide range was demonstrated by means of tables, in which the observed and calculated values of K were compared for a series of tubes in which zymase (*dauerhefe*) and co-enzyme (boiled yeast extract) were employed in varying proportions.

Herzog, from experiments carried out previous to the discovery of the heat-resistance component, came to the conclusion that the fermentation process was to be represented by the formula

$$\frac{K}{K_1} = \left(\frac{C}{C_1}\right)^x,$$

where C and C_1 represent comparative concentrations of zymase and x has any value from 1 to 2. Such a formula would obviously only hold in those cases in which the ratios between zymase and co-enzyme are maintained on a constant basis. The formula which we have developed above gives results closely agreeing with theoretical conclusions in all cases in which sources of experimental error, such as the action of outside enzymes, are eliminated.

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Presentation of a dog ten months after double nephrectomy and replantation of one kidney.

By **ALEXIS CARREL.**

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

The animal presented to the Society underwent the extirpation and replantation of the left kidney and the extirpation of the right kidney ten months ago. He is to-day in excellent health.

The result shows that the perfusion of the kidney with Locke's solution, the interruption of the renal circulation for fifty minutes and the disconnection of the renal nerves with the central nervous system do not produce any lesion of the kidney incompatible with its functions.

18 (356)

A demonstration of the life-saving action of eserin in poisoning by magnesium.

By **DON R. JOSEPH** and **S. J. MELTZER.**

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

At the May meeting of this Society, one of us (J.) reported that by the use of magnesium, certain toxic effects of physostigmin can be completely overcome. In our present communication we wish to bring out the fact that the antagonism between physostigmin and magnesium is mutual, at least to a certain extent. We wish to show an experiment which demonstrates that physostigmin can overcome certain toxic effects of magnesium and thus save the life of a poisoned animal.

Both these rabbits (A and B) received at about the same time 1.2 gram of magnesium sulphate per kilo of body weight. The injections were given intramuscularly in the lumbar region. Rabbit B received in addition one milligram of eserin, also intramuscularly. Rabbit A is already dead. Rabbit B is still alive; although anesthetic and limp, it breathes regularly and apparently is in no danger of death.[1]

[1] By the end of the meeting, rabbit B had recovered completely.

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The mechanical destruction of pepsin.

By **A. O. SHAKLEE** and **S. J. MELTZER.**

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

At various times since 1884, one of us (M.) has studied the effects of shaking upon living cells, such as red blood corpuscles, bacteria, and arbacia eggs. Those experiments led to the general conclusion that shaking, by virtue of the mechanical factor, exerts a profound influence upon living organisms. In the present series of experiments we intend to investigate the changes which shaking may produce in the action of ferments. We began with the study of pepsin, the determination of which is greatly facilitated by several recently described reactions.

Solutions of pepsin, partly filling long bottles, were shaken at room temperature and at a temperature of 33° C. for periods of different lengths, by means of shaking machines. Our results which we state here very briefly are unmistakable. Shaking under these conditions destroys pepsin. Even short periods greatly diminish its strength. If shaken long enough it is completely destroyed. The temperature has a marked influence upon the rate of destruction. Higher temperatures hasten the destruction.

That the effect is not due to oxidation was proved by substituting for the air in the bottles, hydrogen, carbon dioxide or oxygen. There was no appreciable difference in the results.

It was also shown experimentally that the destruction was not brought about by any rise of temperature caused by the shaking. Maximum thermometers were fixed in the bottles perpendicular to their long axes, that is, perpendicular to the direction of shaking: in no case did the thermometer inside register as much as a degree higher than the thermometer outside.

We have also found that the degree of shaking which occurs in the animal body is sufficient to reduce the activity of pepsin. This was determined by introducing a small bottle containing a solution of pepsin into a dog's stomach through an esophageal fistula and permitting it to remain there for 24 hours or longer. The pepsin strength was diminished as much as 40 per cent. com-

pared with that of the pepsin in a similar bottle kept in the thermostat at a temperature of 39° C.

The reported results were demonstrated by the reactions for pepsin of Jacoby and Solms, of Fuld, and of Gross.

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A demonstration of the effects of CO_2 upon the frog's pupil.

By **JOHN AUER.**

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

Frogs placed in an atmosphere of CO_2 gas show within thirty seconds, before any symptoms of excitement, a good constriction of the pupil. This constriction becomes almost maximal within five minutes; there is no dilatation.

The same effect is exerted by CO_2 gas upon excised frog's bulbi.

When the frog's iris, in the excised bulbus or in the living animal, is under the influence of CO_2 gas, the powerful mydriatic effect of adrenalin is strongly reduced.

Since CO_2 produces this myotic action upon excised bulbi, its effect must be exerted, largely at least, upon the sphincter pupillæ, that is, its action is peripheral. These experiments, however, do not exclude a possible central action.

This myotic effect of asphyxia in frogs is interesting, as asphyxia in mammals produces chiefly dilatation.

21 (359)

On the specific acquired resistance of red blood cells.

By **RICHARD WEIL.**

[*From the Loomis Laboratory, Department of Experimental Pathology, Cornell University Medical School, New York City.*]

It is a well known fact that the serum plays a preponderating rôle in the immune reactions of animals. On the other hand, it has been amply shown that the immune characters of the serum

may be in no proportion to the immunity of the organism as a whole. There must therefore be an additional factor, hypothetically designated by Behring as cytogenetic immunity. Of this type of immunity there is the following experimental evidence. In 1898, Kossel stated that if an animal were injected with eel serum, its washed red cells manifested resistance to the hemolytic action of the latter in vitro; a fact confirmed by Camus and Gley, and by Tshistovitch. In 1908, it was shown by Morawitz and Pratt that in animals injected with phenylhydrazin, the red cells became resistant to all hemolytic agents. This they showed to be not an immune reaction, but the direct result of the chemical action of the drug on the red cells.

It was the object of the present series of experiments to determine whether it was possible to induce a *specific* resistance of the red cells to poisons injected into the animal. Dogs and rabbits were used. Among the hemolysins injected were: eel serum, and dog serum; saponin, and digitalin; staphylolysin, tetanolysin, and prodigiosus toxin; and phenylhydrazin. Eel serum, saponin, and phenylhydrazin were selected for routine experimentation. All of these were found to induce a severe grade of anemia. The animals were bled at intervals, and the resistance of the corpuscles, after repeated washings in salt solution, tested against a variety of destructive agents. Eel serum was found to induce occasionally a marked change in the red cells; saponin did so almost invariably, in case the animal survived the treatment; phenylhydrazin did so without exception. In the early stage of treatment, the resistance of the red cells to all hemolytic agents, including the injected hemolysin, was diminished. In the later stages, it was increased in a characteristic manner. The red cells of animals injected with phenylhydrazin showed a marked increase of resistance to all types of hemolysins. Animals injected with eel serum and with saponin came to possess a type of erythrocytes which were very resistant to the specific injected hemolysin, but were almost invariably more easily destroyed than normal control cells by all other hemolytic agencies, including anisotonic salt solutions (demonstration). In those animals injected with eel serum, which failed to develop a specific resistance of the red cells, the serum showed marked antihemolytic powers. It is evident, therefore, that the erythrocytes have developed a specific immunity.

The cause of this resistance has been made the subject of further study. After testing the saponin and eel serum on a variety of red cells, the supernatant fluid was pipetted off, and tested on normal red cells. It was found that the fluid which had been in contact with resistant cells was least hemolytic, and vice versa. It is conceivable that the resistant erythrocytes may either absorb a disproportionate quantity of the hemolysin, or may contain a neutralizing substance.

It has been customary in human pathology to judge of the resistance of red cells according to their vulnerability in anisotonic solutions of salt. The above described experiments indicate that specific resistance to a circulating toxin may be associated with marked loss of resistance to anisotonic solutions of salts. The red cells in advanced cases of cancer have been shown (Lang and others) to possess a greatly increased degree of resistance to anisotonic solutions. The demonstration of a hemolysin in the circulating blood of cancerous cases, and of an increased resistance thereto on the part of the red cells, has made it possible to prove that the resistance is specific to this hemolysin, and only accidental and occasional for the anisotonic solutions.

22 (360)

The butyric reaction for syphilis in man and in the monkey.

By **HIDEYO NOGUCHI.**

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

In a preliminary communication[1] I stated elsewhere that an increase in certain protein constituents of the blood serum and of the cerebro-spinal fluid of patients suffering from active or latent syphilis or parasyphilitic affections is a constant occurrence. I wish to describe here briefly the technique of employing butyric acid for the detection of this increase of protein.

Cerebro-spinal fluid. — One or two parts[2] of spinal fluid[3] are mixed with five parts[4] of 10 per cent. butyric acid solution[5] and are

[1] Noguchi: *Jour. of Exp. Med.*, 1909, xi, p. 84.

[2] 0.1 or 0.2 c.c. are sufficient and convenient.

[3] Must not contain blood.

[4] 0.5 c.c. for the quantities above specified.

[5] Best in 0.9 per cent. salt solution.

heated over a flame to a brief boiling. One part[1] of normal solution of NaOH is then added quickly to the heated mixture and the whole is boiled once more for a few seconds. The presence of an increased content of protein in a spinal fluid is indicated by the appearance of a granular or flocculent precipitate which gradually settles under a clear supernatant liquid. The intensity of the reaction varies greatly according to the amount of the protein which a given specimen contains, but the granular appearance of the precipitate means a positive reaction for syphilis or parasyphilitic affections.

With normal or non-specific specimens there will be a slight opalescence or sometimes a marked turbidity which, however, does not settle out in several hours or even in 24 hours.

Blood serum. One part[2] of clear serum is mixed with nine parts[3] of half saturated solution of ammonium sulphate. Upon complete precipitation, the mixture is centrifugalized and the compact deposit (globulin fractions) is separated from the supernatant fluid by decantation. The deposit is then redissolved in ten parts[4] of 0.9 per cent. salt solution, in which it easily dissolves. The globulin solution thus obtained is ready for the acidification with butyric acid. This is done by mixing one part of the solution with an equal part of 10 per cent. butyric acid solution. It is my custom to take 0.5 c.c. of each solution for mixing. On standing, prompt and dense turbidity begins to appear in the tubes containing the fractions of the serum of syphilitic or certain non-syphilitic patients, while those from normal serum remain quite clear after several hours, or show only slight opalescence without precipitation.

A few words may be added here as to the results of investigations made with the above methods. About 250 specimens of cerebro-spinal fluid, mostly of parasyphilitics, and about 300 specimens of the blood of syphilitic and parasyphilitic patients, together with many control specimens derived from patients with non-syphilitic diseases and normal persons, have been studied.

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[1] Namely, 0.1 c.c. in this case.

[2] Usually 0.5 c.c. is sufficient and convenient.

[3] Namely, 4.5 c.c. in this instance.

[4] 5 c.c. in this instance.

Spinal fluid derived from parasyphilitic cases gives a typical reaction, becoming granular in a few minutes and sedimenting in from 10 to 15 minutes. Cerebro-spinal fluid from cases of congenital, tertiary or secondary syphilis gives quite constantly a positive reaction, but the intensity is usually less and two hours may be required before the characteristic granular appearance becomes manifest. Cerebro-spinal fluid from cases of cerebral or spinal syphilis gives invariably a positive reaction. Negative reaction was obtained with the spinal fluid from cases of acute anterior poliomyelitis, epilepsy, alcoholic psychosis, dementia precox, senile dementia, spastic paraplegia, lobar pneumonia and typhoid fever. On the other hand, an abundant flocculent precipitate was usually formed with the spinal fluid from cases of tubercular meningitis, influenza meningitis, or epidemic cerebro-spinal meningitis.[4] Cerebro-spinal fluid collected from two cases of hydrocephalus also gave abundant precipitation. In all of these acute inflammatory cases, except one of hydrocephalus, the Wassermann reaction was, however, negative. A number of post-mortem spinal fluids were examined with such results that it seems desirable to use the method as a routine diagnosis for syphilis or parasyphilitic affections at autopsy. In the spinal fluid of two monkeys with active experimental syphilitic lesion at the site of inoculation, which persisted about 6 months, the reaction was positive.

Referring to the results of examinations of the blood serum, it appears that the reaction is non-specific for syphilis, because a similar reaction can be obtained in certain cases of tuberculosis, carcinoma and Hodgkin's disease.

In view of the constancy with which an abnormally high globulin content attends the florid stage of syphilis and appears to be present in an early primary stage, and is present in the late secondary and tertiary stages of imperfectly treated cases, one is thus enabled to follow the course of an anti-syphilitic treatment. Moreover the butyric acid test is a more delicate indicator than the Wassermann reaction, for the latter is very frequently negative in this latter class of cases. Under conditions of adequate treatment, the globulin fraction of the blood serum is not increased. A negative re-

[4] These acute inflammatory conditions are quickly and perfectly excluded by clinical and usual microscopical methods of diagnosis.

action with the butyric acid test indicates either the absence of syphilitic infection or a successful cure of the disease. There is no necessary relation between the Wassermann test and the quantity of globulins in the luetic serum.

23 (361)

The quantitative separation of leucin from valin.

By **D. D. VAN SLYKE** and **P. A. LEVENE.**

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

Of the known amino-acids determined in semi-quantitative estimations of final proteolytic products, leucin and its relatives, isoleucin and valin, have proven unusually difficult to prepare pure in even approximately quantitative amounts. The separation of these substances, because of their close physical and chemical similarity, has offered almost insurmountable difficulties to previous investigators. The acids form isomorphous mixtures which are absolutely inseparable by crystallization; and their esters have so nearly the same boiling points that they cannot be fractionated by distillation. Because of these difficulties, most investigators have not attempted to separate the mixture, but have reported the entire mass as leucin. Fischer[1] states that all the figures reported from his laboratory for leucin in protein hydrolyses refer to this mixture. Ehrlich[2] has recently reported a method for separating the three substances, but it involves a long process, large losses, and the racemization of the isoleucin and valin.

We have been able to separate the leucin isomers readily from valin in quantitative amounts. The method, which is very simple, rests on the fact that if a molecular lead acetate solution is added to an ammoniacal solution of the leucin-valin mixture, the leucins are precipitated as analytically pure $Pb(C_6H_{12}O_2N)_2$. If too great an excess of lead acetate is added, a portion of the valin may also be precipitated. Consequently, the mixture is first analyzed, an estimate of the proportion of leucin calculated from the carbon content, and 20 per cent. excess of the theoretical amount of lead

[1] Fischer: Unters. über Aminos., Polypeptide, und Proteïne, p. 67.

[2] Ehrlich: *Bioch. Zeitschr.*, 8, 399, 1908.

acetate used for precipitation. The valin is obtained analytically pure by freeing the filtrate from lead with H_2S, evaporating to dryness, and washing with absolute alcohol. A slight amount of valin dissolves, but is regained by evaporating the washings.

The following is a typical separation, the material being a portion obtained by tryptic digestion of casein and fractional distillation of the amino-acid esters. 12.546 g. of the mixture was used. Analysis showed 52.79 per cent. C, 9.55 per cent. H. The mixture was suspended in 80 c.c. of boiling water. The flask was removed from the flame and 20 c.c. of concentrated aqueous ammonia added. The flask was loosely stoppered, and shaken gently until the acids were dissolved. The leucin was then precipitated with 25 c.c. of M/1 lead acetate. The cooled solution was filtered, and the precipitate washed with 50 c.c. of dilute ammonia. 8.955 g. of lead salt, equivalent to 5.025 g. of leucin, and 7.322 g. of valin were obtained analytically pure, making 12.347 g. from the original 12.546 g. Analytic data:

Lead salt: (1) 44.25 per cent. Pb; (2) 44.36 per cent. Pb. Calculated for $Pb(C_6H_{12}O_2N)_2$, 44.29 per cent. Pb.

Valin: 51.44 per cent. C; 9.42 per cent. H. Calculated for $C_5H_{11}O_2N$, 51.24 per cent. C; 9.47 per cent. H.

The specific rotation of the valin was $[\alpha]^{20}D + 26.51°$. The pure active substance has the rotation + 28.8°. The product was partially racemized, the usual result of long tryptic digestion.

The lead-leucin salt contains a mixture of leucin and isoleucin. Levene and Jacobs[1] have shown that these isomers can be readily separated in the absence of valin. The complete separation of the two leucins from valin, therefore, renders the systematic separation of all three comparatively easy of accomplishment. This is of importance, not only for protein analysis, but also for the preparation of pure active valin and isoleucin, a task which has hitherto been extremely difficult.

The work will be reported in full in the *Biochemische Zeitschrift.*

[1] Levene and Jacobs: *Bioch. Zeitschr.*, 9, 231, 1908.

24 (362)

Further studies on the constitution of inosinic acid.

By **W. A. JACOBS** and **P. A. LEVENE.**

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

In a former article[1] on the constitution of the inosinic acid obtained from beef extract, we have demonstrated that by acid hydrolysis there is formed an intermediate product, a pentose phosphoric acid, which we isolated as a well crystallized barium salt. From the fact that this body showed strong reducing properties, it is evident that the aldehyde group is free, and the phosphoric acid is bound, ester-like, on one of the hydroxyl groups of the pentose. As the inosinic acid itself does not reduce Fehling's solution, it is at once obvious that the hypoxanthin contained in its molecule must be bound as in a glucoside on the aldehyde group. We also mentioned that upon alkaline hydrolysis we were able to isolate a small quantity of a silver compound of a purin-pentose complex which gave all the qualitative tests for such a body.

Meanwhile it came to our notice that Haiser and Wenzel[2] had obtained a compound of hypoxanthin and a pentose from karnin to which they gave the name *inosin.* We have succeeded, by heating the barium salt of inosinic acid in water solution in a sealed tube at 125°–130°, in obtaining a mixture from which we have isolated a substance which in all respects corresponds with Haiser and Wenzel's inosin.

From this substance we obtained a levorotatory pentosazone. Furfurol distillation yielded the phloroglucid required by a pentose.

[1] Levene and Jacobs: *Berichte d. deut. chem. Gesell.*, 41, 2703 (1908).

[2] Haiser and Wenzel: *Monatshefte für Chemie*, 29, 157 (1908).

25 (363)

The significance of changes in the permeability of the plasma membrane of the living cell in the processes of stimulation and contraction.

By **RALPH S. LILLIE.**

[*From the Physiological Laboratory, Zoölogical Department, University of Pennsylvania.*]

The general facts indicating that stimulation is dependent on a temporary increase in the permeability of the surface layer or plasma membrane of the irritable element are as follows:

A. The nature of the motile process in such plants as *Mimosa*, *Dionæa* and the Cynareæ, where the movement depends on a sudden loss of turgor. Such a change indicates either (1) a sudden decrease in the concentration of the osmotically active substances within the cell due to chemical action, or (2) a sudden loss of impermeability relatively to the osmotically active substances. The latter explanation is almost certainly the correct one.

B. The identity of the electrical change accompanying stimulation in motile plant cells with that observed in irritable animal tissues (Burdon-Sanderson), indicating a fundamental similarity in the conditions of stimulation in the two classes of organisms.

C. The fact that the post-mortem increase in permeability is accompanied by contraction in muscle cells; the same is, of course, true of motile plant organs where the movement depends on loss of turgor.

D. The nature of the electrical change accompanying stimulation. If the irritable element represents a concentration-cell in which a semi-permeable membrane (the plasma membrane) greatly diminishes the velocity of the anion, while leaving that of the cation practically unaltered (Ostwald-Bernstein membrane theory), any marked increase in permeability relatively to the anion must result in a fall of the potential difference between exterior and interior of the irritable element. Such an electrical change actually occurs on death or injury of the element; also momentarily during stimulation. A demonstrable increase in permeability occurs at death; inferentially, therefore, the same change occurs during stimulation.

E. The fact that an irritable tissue loses irritability for a variable usually brief) period after stimulation (refractory period), — indicating that at that time the property of semi-permeability, on which electrical stimulation depends (Nernst), — is temporarily lost.

The special observations presented in this paper are as follows:

1. *Arenicola* larvæ are stimulated intensely by pure isotonic solutions of various salts (NaCl, KCl, NH_4Cl, LiCl, $SrCl_2$, $BaCl_2$), contracting to half their length for several seconds when first introduced into the solution. At the same time a yellow pigment contained in the cells of the organism diffuses freely to the exterior and colors the solution.

2. Solutions which do not produce this strong initial contraction do not cause such loss of pigment. Isotonic $CaCl_2$ and $MgCl_2$ (especially the latter) are instances. In these solutions the muscles lose the power of contraction and the organism becomes stiff and motionless (though still propelled by the cilia which remain active). Addition of small quantities of $CaCl_2$ to a NaCl solution prevents the strong initial contraction and loss of pigment (antitoxic action).

3. $MgCl_2$ and similarly acting solutions appear to *decrease* the permeability of the tissues, and so prevent the ionic transfer on which stimulation depends. The general action of anæsthetics consists in *decreasing the normal permeability;* stimulating agencies, on the other hand, have the reverse effect.

4. Strong solutions of fat-solvents (chloroform, ether, benzol, (etc.) produce a contraction of the muscles accompanied by loss of pigment, even in $m/2$ $MgCl_2$. This effect is to be referred to an alteration of the lipoid substances in the plasma membrane. Such alteration, if slight, *decreases* permeability (anæsthetic action in low concentrations); if extreme, it produces the reverse effect, with resulting stimulation.

5. The hypothesis is presented that the chemical effect of the above changes in permeability depends essentially on their influence in varying the rate at which carbon dioxid leaves the cell. The velocity of the oxidative energy-yielding processes whose end-product is CO_2 is thus varied with the rate of removal of this latter substance from the system; this velocity is accordingly increased during the increased permeability of stimulation, and is decreased

during anesthesia or inhibition. This view is supported by a consideration of the electrical changes accompanying inhibition and stimulation, respectively ("positive" and "negative variations").

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On the relative concentration of lysins, precipitins, agglutinins, opsonins and related substances in the different body fluids of normal and immune animals.

By **F. C. BECHT** and **J. R. GREER**. (By invitation.)

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

The present work was suggested by some of our previous work with Prof. Carlson on the physiology of lymph. It was suggested that it would be of profit to establish the differences between serum and the other body fluids in their content of antibodies of various kinds in normal and immune animals, with the hope that it would have some bearing upon the problem of lymph formation, and also upon the origin of these antibodies. Thus far the hemolysins, hemagglutinins, bacterial agglutinins, bacterial opsonins, hemopsonins, and precipitins have been studied in the serum, neck lymph, thoracic lymph, pericardial fluid, cerebrospinal fluid, and aqueous humor. The bacterio-lysins are also under consideration.

The results with normal dogs have been the following: Hemolysins are found in the serum, thoracic lymph, and neck lymph in the normal animal. Serum and thoracic lymph contain them in almost equal quantities, with a slight balance in favor of the serum. The hemolytic power of the neck lymph is much lower than that of the serum, and is almost entirely wanting in the lymph which is secured without massage. In two of seven cases there was a small amount of hemolysis in the pericardial fluid, in the remaining cases there was no laking when the fluid was free from erythrocytes. There is no hemolysis in the cerebrospinal fluid, except in one case where there was a trace of free hemoglobin. There was no hemolysis in the aqueous humor. The hemagglutinins run parallel with the hemolysins except that they act in higher dilutions than the latter.

Bacterial agglutinins are found in the serum, thoracic lymph, and neck lymph. The two lymphs were of equal strength, and the serum was approximately ten times as strong. Pericardial fluid, cerebro-spinal and aqueous humor were entirely lacking in these anti-bodies. No precipitins for rabbit serum are found in any of the body fluids of the normal dog.

The hemolytic power of the serum and thoracic lymph of dogs immune to typhoid is slightly higher than that of the same fluids of the normal animal. No increase has been noted in the other fluids examined. The hemagglutinating power is increased in the serum, neck lymph, thoracic lymph, and pericardial fluid, particularly in the latter. The bacterial agglutinating power of the serum, neck lymph, and thoracic lymph is much increased. The pericardial fluid and aqueous humor are inactive or at most show only a trace. The cerebro-spinal fluid has not yet been tested.

In dogs rendered immune to rabbit blood we have not yet noted any very marked increase in hemolysins over those of the normal animal. There was a marked increase of the hemagglutinins in the serum, neck lymph and thoracic lymph, and in one case in the pericardial fluid and aqueous humor. Immunization to rabbit blood has no effect on the bacterial agglutinins. The injection of defibrinated rabbit blood did not produce precipitins for rabbit serum in any of the body fluids of the animal. The work on bacterial opsonins and hemopsonins in both normal and immune animals has been entirely unsatisfactory.

The work is being continued and extended to cats and rabbits. Later, lymph will be collected from various organs and extracts from various tissues made with the hope of finding the source of these antibodies. Parallel experiments are being made on the number and kind of leucocytes in the various body fluids.

27 (365)

Studies of the influence of various dietary conditions on physiological resistance. I. The influence of different proportions of protein in the food on resistance to the toxicity of ricin and on recuperation from hemorrhage.[1]

By **NELLIS B. FOSTER.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons.*]

It was planned to compare, in these experiments, the behavior of two sets of dogs under variously induced pathological conditions. The experiments were conducted on two pairs of dogs, each pair being kept under identical conditions, so far as they could be controlled, except with respect to the food. One animal of each pair was given *liberal* amounts of protein in the daily diet, the other received *barely sufficient* protein to provide for the necessary nitrogenous metabolism, the remaining ingredients of the food for each animal being uniformly equal to the daily amounts ordinarily given per kilo, in this laboratory, to perfectly healthy dogs. No attempt was made to maintain equal caloric values in the diets. In each experiment the animal was fed on a diet of hashed lean meat, cracker meal and lard, the meat being gradually increased or diminished to a high or low plane, according to the plan in each case and before the particular pathological condition was induced.

It was a part of the plan of the work to keep the animal in each instance on a high or low plane of protein nutrition for a *considerable period* before the pathological phase was brought into the experiment. Such a course is not only desirable but essential, for if, as has been claimed, a diet rich in protein exercises a dele-

[1] This study was begun during the summer of 1905, at Dr. Gies' suggestion and has been carried forward from time to time under his direction and with the aid of a grant from the Rockefeller Institute. I am also indebted to Dr. Flexner for important suggestions.

The work has been frequently interrupted by researches in other directions and has been beset by unusual experimental difficulties. Although planned to be the first of a series of investigations, in point of publication it is the second from this laboratory on the general subject stated above. See Dissertation by Welker, Columbia University, 1908. It is Dr. Gies' intention to continue investigation along these lines.

terious influence on the animal organism, that effect is not ordinarily an immediate one, but rather the slow perversion of organic function induced by chronic misuse. Again, if benefit results from a special diet under any condition, time may be required to make that benefit clearly distinguishable. The dogs used in these experiments were under daily observation for periods of at least two months and in several cases for four months before inauguration of the pathological phase.

In testing the physiological resistance of the dogs in these experiments, use was made of two methods: (1) hypodermic injection of the toxin, ricin; and (2) the withdrawal of definite amounts of blood from one of the large arteries. Ricin was selected because it produces some symptoms which are analogous to those of acute infections, namely, fever, cardio-vascular embarrassment and marked prostration. As in the case of infections, ricin also causes noticeable stimulation of metabolism, as is shown by the increased elimination of nitrogen and sulfur after its injection. The disadvantages in its use are that immunity quickly arises and that the material is so very toxic that no latitude is permitted for minor individual peculiarities in animals. By experiment 1 milligram per kilo was found to be the maximum nonlethal dose for the commercial sample used.

EXPERIMENTS WITH RICIN.

Six dogs were subjected to inoculation with ricin. Of these, three were on a high plane of protein nutrition, the daily amount of food containing uniformly in each case 1.4 to 2.0 grams of N per kilo of body weight. After inoculation with ricin (1 mg. per kilo), all of these three specially well-fed animals died. A fourth dog was on a medium plane of protein nutrition, 1.1 gram. of N per kilo, and this dog survived the inoculation. Of the two remaining animals of this group, both on a low plane of protein nutrition, one died and one survived. The dog which survived received the protein equivalent of 0.35 gram of N per kilo of body weight during the experimental period and the one that died, 0.37 gram of N per kilo.

In order to test the effect of exercise, the former of these two dogs, after the completion of the above mentioned experiment, was

allowed to run at large in the laboratory for six months (July 17, 1906, to Jan. 10, 1907), no effort being made to collect excretions for analyses. During this time the protein content of the diet was gradually dropped to an equivalent of 0.27 gram of N per kilo. The animal was painfully thin but appeared to be in excellent health. She was lively during the warm months of summer and autumn, but with the advent of colder weather she became less and less active and lost weight, although the laboratory was adequately heated. It finally became evident that the diet must be changed. The portion of meat in the diet was gradually increased, but a diarrhea supervened, which proved fatal.

Hemorrhage Experiments.

Although hemorrhage is one of the crudest factors for the determination of physiological resistance, it has, nevertheless, the advantage of being a real test, inasmuch as the production of new blood is an exemplary reparative process. Hemorrhage, unlike the injection of ricin, can also be gauged, to some degree at least, to meet the individual resistance of the animal, as I have found in these experiments. The procedure was uniform throughout this part of the work. At the first hemorrhage from each animal, the endeavor was made to take blood equivalent to 4 per cent. of the body weight. After an interval of four days this procedure was repeated; and again eight days after the initial hemorrhage, making three hemorrhages at successive intervals of four days. All operations were conducted under ether narcosis.

Six dogs were experimented on in this manner, three upon high, three upon low, planes of protein nutrition, and four survived. Of the two which died, one had been on a low plane of protein nurition (0.4 gram of N per kilo) and one on a high nitrogen plane (1.4 gram per kilo). Control animals on more extreme dietary conditions, withstood the effects of greater hemorrhages. Therefore, it is evident, I think, that these two deaths must be assigned to reasons outside the realm of this research. In the case of the dog on the high plane of protein nutrition, the cause is not far to seek; the dog was a collie, apparently pretty well bred, and it is a matter of common knowledge that these dogs have very low resistance. The dog that died on a low plane of protein nutrition was a fox terrier mongrel.

I. *Outlines of the Experiments.*

Dog No.	N of food per kilo, grams.	Nature of the experiment.				Result.
		Ricin.	Hemorrhage.*			
		1 mg. per kilo of body weight.	Per cent. of body weight.			
			1	2	3	
I	0.35	+	...	...	...	Very sick : Survived.
II	1.10	+	...	...	...	Survived.
III	0.37	+	...	...	...	Died.
IV	1.5	+	...	...	...	Died.
V	3.37 —for 1 mo. 0.51†—for 20 ds.	+	...	...	...	Over fed : Died.
VI	0.40	+	...	...	...	Died.
VII	0.44	...	3.3	2.5	...	Died.
VIII	1.57	...	4.0	4.3	3.1	Survived.
IX	1.4	...	3.8	2.3	0.6	Died.
X	0.35	...	4.2	4.0	4.0	Survived.
XI } Twins.	0.81	...	3.6	3.4	3.7	Survived.
XII } Twins.	0.23	...	4.2	3.0	2.9	Survived.

* The numerals above the figures for percentage indicate the number of the hemorrhage in the series applied to the dog in question.

† The food was dropped to this equivalent at once after two days of fasting following pronounced gastro-intestinal disturbances.

II. *General Summary.*

Totals (I — XII)	High plane of protein nutrition — 6 dogs	Survived, 2	
		Died, 4	
	Low " " " 5 dogs	Survived, 2	
		Died, 3	
Ricin experiments	High plane of protein nutrition — 3 dogs	Survived, 0	
		Died, 3	
	Low " " " 2 dogs	Survived, 1	
		Died, 1	
Hemorrhage experiments	High plane of protein nutrition — 3 dogs	Survived, 2	
		Died, 1	
	Low " " " 3 dogs	Survived, 2	
		Died, 1	

In order to reduce individual idosyncrasy to its lowest terms the last experiment of the series was conducted on two dogs from

the same litter. These animals were half-breed Scotch terriers, about two years old, that had lived together under the same conditions until they were brought to the laboratory. One was gradually accustomed to a diet containing only 0.2 gram of N per kilo, the other to food containing 0.8 gram of N per kilo. As a matter of further interest the opsonic index of these dogs was several times estimated, using *Staphylococcus aureus* that had been rendered pathogenic to dogs. This index was, and remained, the same for both animals. The final hemorrhages were survived by both animals and, so far as one might estimate, about equally well.

The accompanying tables present an outline of the experiments and show details not mentioned above.

Thirty second meeting.

New York University and Bellevue Hospital Medical College. February 17, 1909. President Lee in the chair.

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A method for the direct observation of normal peristalsis in the stomach and intestines.

By **YANDELL HENDERSON.**

[*From the Physiological Laboratory of the Yale Medical School.*]

When the abdomen is opened peristalsis ceases. Because of this fact all previous investigators have found difficulty in making observations directly upon the motility of the stomach and intestines. The peristalsis which has been seen differs considerably from that which the radiographs of Cannon have shown to be the normal movements.

Failure of peristalsis, as Meltzer especially has pointed out, is one of the characteristic and important phenomena of surgical shock. I have advanced the hypothesis[1] that the cause of shock is acapnia. By applying this theory to the problem of maintaining normal peristalsis after laparotomy, the following simple and effective method was devised. After the administration of a moderate dose of morphin, the animals (dogs of about 10 kilos) were anæsthetized with chloroform. So far as possible the hyperpnœa of the initial stage of anæsthesia was avoided. The abdomen was laid open the entire length of the mid line. The omentum was cut out, and the viscera moved sufficiently to bring into view the upper colon, lower ileum, and the greater curvature of the stomach from the pre-antral groove to the pylorus. A sheet of transparent celluloid was inserted under the body wall and over the viscera; and the air in the space back of this window was washed out with a stream of carbon dioxide gas. To the trachea was attached a tube 15 mm. in diameter and 2 meters in length. Blood gas analyses showed that the blood-gases were thus main-

[1] Y. Henderson: *American Journal of Physiology*, 1908, xxi, 126.

tained normal, — not asphyxial in respect either to the oxygen or carbon dioxide contents. When the stomach was distended with air, and the large intestine and lower ileum with bread mush, movements in these three parts of the alimentary canal were seen identical with those shown by the radiographs of Cannon. In the stomach a deep constriction developed at the pre-antral groove every 15 seconds and moved toward the pylorus where it disappeared as its successor was developing. In the colon there was active anti-peristalsis. In the ileum vigorous rhythmic segmentation was seen.

In other experiments I have found that animals under ordinary operative conditions develop, and remain in, a state of acapnia. This lowered carbon dioxide content of the blood and tissues, by inducing loss of tonus, is the cause of the failure of peristalsis after laparotomy. The essential point in the above described method is the prevention of acapnia.

29 (367)

Studies on the effects of carbon mon-oxide poisoning.

By **A. I. RINGER.** (By invitation.)

[*From the Physiological Laboratory of the New York University and Bellevue Hospital Medical College.*]

If an animal be allowed to breathe an atmosphere containing carbon mon-oxide, it will soon present a series of circulatory, respiratory, cerebral and metabolic disturbances, which, if carried too far, will result in death. These disturbances are believed to be brought about by the reduction of the oxygen-carrying capacity of the blood, due to the formation of the relatively stable carbon mon-oxide-hemoglobin, thus producing a state of progressive asphyxiation of the tissues. The severity of these disturbances depends entirely upon the degree of asphyxiation; and, with the exception of some individual peculiarities in a few of the twenty-one dogs that I have experimented upon under anæsthesia, all presented the same symptoms at the same stage of asphyxiation.

In nine experiments the following subjects were studied in their relationship to the degree of saturation of the hemoglobin with carbon mon-oxide: (1) the pulse, (2) the blood pressure,

(3) the number of respirations per minute, (4) the volume of air respired per minute; in twelve experiments: (1) the physical symptoms, (2) the point of onset of coma, (3) the point of death.

As soon as the animal begins to breathe the carbon mon-oxide there is an immediate acceleration of the pulse, which steadily gains in frequency until 45–50 per cent. of the hemoglobin is saturated with carbon mon-oxide. Then the pulse rate is at its maximum. After that it declines gradually until a point is reached between 71–74 per cent. of carbon mon-oxide saturation, when the pulse rate falls abruptly and the heart ceases to beat.

The blood pressure in about half the cases was found to go gradually downward in spite of the markedly increased rate in the heart beat. In the rest of the cases there was a preliminary rise in pressure of about 10–15 mm. of Hg, which reached its maximum when 45–50 per cent. was saturated with carbon mon-oxide. This was followed by a gradual decline, until when about 67 per cent. of the hemoglobin was saturated, there was an abrupt fall in blood pressure.

The number of respirations per minute, taking two characteristic examples, was found to increase from a normal of 19 and 22 to 32 and 45, respectively, when about 50 per cent. of the hemoglobin was saturated with carbon mon-oxide. In other cases there was no increase in the rate of respiration at all, but there was a marked increase in the depth of each inspiration. The volume of air respired per minute, however, increased in all cases as soon as the animal began to breathe the carbon mon-oxide. It presented a curve with an ascending and descending limb. The maximum ventilation of the lungs took place when about 45 per cent. of the hemoglobin was combined with carbon mon-oxide. After that it declined gradually. At about 65 per cent. saturation, however, breathing became irregular, sometimes of the Cheyne-Stokes variety. Respiration always stopped about one to three minutes before the heart ceased beating.

When about 40 per cent. of the dog's hemoglobin is deprived of its oxygen carrying capacity, the animal begins to get weak. It cannot stand on its legs. It is in a state of general indifference. It does not partake of any food or drink, though it may be hungry. It is seized with vomiting and occasionally has convulsive spells.

It responds to a call very slowly, and sometimes falls into a sleep, from which it is aroused with difficulty. Actual coma sets in at a point immediately following the decline in the pulse rate, or when about 50 per cent. of the hemoglobin is saturated with carbon mon-oxide. This has been found to be constant in all cases.

Death also takes place in all dogs at about the same point of saturation. Not a single one reached the 75 per cent. mark. All died between 71 and 74 per cent. of saturation of the hemoglobin with carbon mon-oxide. Neither size, weight nor strength seemed to influence the point of death.

From the foregoing it is seen that the symptoms of carbon mon-oxide poisoning may be conveniently divided into three stages: First or Compensatory Stage, which lasts up to 50 per cent. saturation; second or Stage of Depression which lasts up to about 70 per cent. saturation; third or Stage of Collapse.

SYMPTOMS OF THE FIRST OR COMPENSATORY STAGE.

1. Gradual increase in pulse rate.
2. High blood pressure.
3. Spasmodic attacks of vomiting.
4. Slight dyspnœa at first — more marked at the end.
5. Muscular weakness.
6. Drowsiness, indifference and deep sleep from which dog can be aroused.

SYMPTOMS OF THE SECOND OR STAGE OF DEPRESSION.

1. Ushered in by clonic convulsion and muscular rigidity which lasts for a few minutes.
2. Deep narcosis from which animal cannot be aroused.
3. Pulse declines gradually in rate, tension and volume.
4. Respiration either rapid and shallow, or slow and deep; it is more or less regular.
5. Absolute loss of sensation.

SYMPTOMS OF THE THIRD OR STAGE OF COLLAPSE.

1. Pulse slow, irregular and of low tension.
2. Respiration irregular.
3. Loss of tone of sphincters.
4. Conjunctival reflex lost.
5. Death due to respiratory paralysis.

Typical experiment of first series.— February 19, 1908. Dog's weight 9.7 Kg. Ether anæsthesia.

Time.	Respiration.	Pulse.	Blood pressure in mm. of Hg.	Volume of air (in c.c.) respired per minute.	Per cent. of Hb saturated with CO.
2.15 P. M.	22	140	90	1,750	
2.18 "	1 per cent. CO respired				
2.35 "	24	148	90	1,925	
2.50 "	36	164	92	2,000	20
3.05 "	40	180	96	2,125	
3.22 "	45	204	104	2,250	47
3.35 "	41	194	94	2,250	
3.45 "	40	166	88	2,100	54
3.50 "	36	120	72	1,925	
3.55 "	32	82	62	1,650	66
3.58 "	8 irregular	56	40	1,175	
4.05 "	10 "	68	—	750	73
4.08 "	Death				

Typical experiment of second series.— March 2, 1908. Dog's weight 6.6 Kg.

Dog placed in air-tight cage through which the gas mixture was driven by means of bellows, operated by a water pressure engine. The ventilation of the cage was 5,000 c.c. of air per minute.

Time.	Respiration.	Pulse.	General condition.
10.30 A. M.	16	104	Dog in good condition.
10.35 "	0.4 per cent. CO respired		
11.00 "	16	104	Dog comfortable and quite active.
11.45 "	16	116	" " " " "
12.00 "	18	124	" " " " "
12.30 P. M.	17	140	Lies quietly; slightly drowsy.
12.45 "	21	170	Drowsiness marked.
1.00 "	18	182	Slight convulsion and vomiting.
1.20 "	16	208	Responds to sensory stimulus after long latent period.
1.35 "	16	192	Convulsions of clonic type.
1.55 "	17	196	Slightly comatose; when called he only opens his eyes.
2.05 "	16	180	Coma quite deep.
2.30 "	—	166	All muscles and sphincters relaxed.
2.45 "	—	—	Taken out of cage. Blood sample shows 52 per cent. of the Hb saturated with CO. Dog placed in warm place near radiator.
3.45 "			Respiration slow and deep, 10–12 per minute.
4.00 "			Dog began to move about.
4.25 "			Able to stand up and walk but falls frequently, due to lack of coördination.
5.10 "			Walked about fairly well, but very slowly.
Next morning			Dog perfectly well.

Experiments have also shown that a dog with as much as 69 per cent. of hemoglobin tied up with carbon mon-oxide which corresponds to the end of the second stage, can be resuscitated if proper treatment be instituted promptly.

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Intestinal excretion during diarrhea.

By **GEORGE B. WALLACE** and **HUGO SALOMON.**

[*From the Laboratory of the Von Noorden Clinic, Vienna.*]

Analyses were made of the fæces of a number of patients with diarrheas of different origin. During one period of observation the patients were on the Schmidt-Strassburger diet, during a second period the diet consisted of 250 grm. sugar daily. In those cases where there was present an ulcerative process in the intestine — tuberculosis, carcinoma — the amount of nitrogen in the fæces was markedly increased — being from 1.7 to 4. grm. daily on the sugar diet. In cases of severe catarrhal inflammation it was not over 1.5 grm.; in light catarrh it was within normal limits. The fat and carbohydrate elimination showed no such striking differences although it was highest where an ulcerative condition was present. Of the inorganic constituents the alkali excretion was fairly parallel to that of nitrogen. The other inorganic constituents were increased by the ulcerative processes but in some instances were increased equally where ulcerations were absent.

The most striking result of the analyses is the high nitrogen excretion which occurs in ulcerative processes in the intestine.

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The vascularity of the kidney as influenced by sensory impulses.

By **R. BURTON-OPITZ** and **DANIEL R. LUCAS.**

[*From the Physiological Laboratory, Columbia University.*]

Quantitative determinations of the blood-flow through the left kidney were made with the aid of the stromuhr of Burton-Opitz. On stimulation of the central end of the sciatic nerve, a slight de-

crease in the vascularity of this organ was observed. This decrease apparently followed a tonic contraction of the blood vessels and not a true constriction as is produced, for example, by stimulation of the corresponding splanchnicus major. It seemed to be merely a tonic reaction of the kidney against the high systemic blood pressure which follows stimulation of the sciatic.

Similarly, the application of cold compresses across the back in the region of the kidneys, reduced the blood-flow through this organ, while hot compresses increased the flow. As the temperature of the organ itself, or of the tissues in its immediate vicinity, was not changed by the compress, these variations in the vascularity of the kidney must have been produced reflexly.

Stimulation of the distal ends of the vagi, below the point where the cardiac branches are given off, did not change the blood flow. The vagus, therefore, appears to carry no efferent vasomotor impulses to the kidney.

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The influence of temperature on hemolysis in hypotonic solutions.

By **PAUL A. LEWIS.**

[*From the Antitoxin Laboratory of the Massachusetts State Board of Health.*]

Hemolysis in hypotonic solutions is progressively increased as the temperature is decreased from thirty seven degrees centigrade to five degrees centigrade. In order to bring out this fact, that modification of Hamburger's method for testing the resistance of erythrocytes, which was introduced by Theobald Smith, was used. The solutions were brought to the required temperature and then the corpuscles were added. The differences are present both at the points of beginning and complete hemolysis, but are only well marked at the intermediate points. This accounts for results obtained by Hamburger (1887 and 1903) who held that temperature within these limits was without influence.

The effect of temperature is the same whether sodium chloride or cane-sugar is used to give tonicity to the fluid. The corpuscles

of the horse, rabbit, guinea-pig, calf, and sheep are equally affected. The differences become well marked after a few minutes' exposure to the different temperatures, and thereafter bringing them to one temperature fails to equalize the hemolysis even after many hours. The effect, then, is on the corpuscle rather than on the surrounding fluid and is exerted chiefly in the first moments of exposure.

Temperatures above 37° C. act variously according to the particular species whose blood is used. Horse corpuscles give distinctly more hemolysis at 42° C. than at 37° C. The corpuscles of the guinea-pig and the calf give still less hemolysis at 43° C. than at 37° C.

33 (371)

A carcinoma of the rat (Flexner-Jobling) considered from the standpoint of immunity.

By **F. P. GAY.**

[*From the Laboratory of the Cancer Commission of Harvard University.*]

Experiments have been in progress for the last year and a half with the Flexner-Jobling rat tumor for the purpose of gaining some insight as to the normal and artifically produced conditions of resistance to this tumor.

The tumor as originally described by Flexner and Jobling was a sarcoma and later became carcinomatous in structure. It has shown no marked variations in histological structure during the eight generations which we have cultivated it. White rats from different dealers varied considerably in their susceptibility to inoculation with this tumor. Animals from the most susceptible source gave 100 per cent. of "takes" whereas the next most susceptible strain gave only 50 per cent. Following inoculation into the region of the axilla metastases occur regularly in the lungs but rarely in the adjacent lymph-nodes. The time of occurrence of metastases would seem to be relatively constant in the most susceptible rats. Metastases occur later and at more irregular intervals in less susceptible animals.

The tumor may be transplanted from the metastases and such "metastatic" tumors would seem after several generations to have

become somewhat more "virulent" in that such tumors grow more rapidly and produce more extensive metastases of a more epithelial type.

Animals that have failed to take the first inoculation of tumor are very seldom susceptible to a second or third implantation. The blood serum of such refractory animals gives no reaction of fixation with cancer extract, and when injected simultaneously with cancer in susceptible rats leads to no prevention of the growth of the tumor. The refractory blood, however, when injected previously to or at the same time with the tumor in naturally insusceptible rats gives a larger percentage of "takes" than in control animals.

If a tumor is removed during the "premetastatic" period a second implanted tumor seldom grows. Subsequent to this period a second implanted tumor does grow.

When a primary growing tumor is left and a second implanted during the premetastatic period, not only does the second tumor fail to grow but the first tumor entirely disappears in many instances. In cases in which the resorption of original tumor was incomplete, from lack of sufficient time the primary tumor showed regressive changes in the nature of cell degeneration or a marked increase of connective tissue stroma.

A reaction of fixation was found with the blood of a few animals with tumor during the premetastatic period but never during metastatic period. The premetastatic period then would seem to be characterized by an active defence on the part of the animal body and during this period reimplantation of tumor increases this resistance to such an extent that the original tumor is destroyed. When this period is passed metastases occur and a second implanted tumor grows.

34 (372)

Influence of temperature upon pepsin.

By **A. O. SHAKLEE.**

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

While studying the destruction of pepsin by shaking pepsin solutions it was thought essential to make some study of the spon-

taneous deterioration that was seen to take place in solutions standing at corresponding temperatures, and also at body temperature.

One per cent. solutions of a commercial pepsin in hydrochloric acid (0.1 per cent., 0.25 per cent. and 0.5 per cent.) were kept in glass bottles at approximately the following temperatures: 5° C., 20° C., 33° C., 37° C. From time to time samples were tested by Fuld's method, modified somewhat to decrease the error. The following results were obtained for solutions in 0.25 per cent. hydrochloric acid at 37° C.:

Duration of heating in days.	*Destruction.*	$\frac{x}{t(1-x)}$
0.5 days.	19.5 per cent.	.48
0.84 "	31 "	.53
1.0 "	35 "	.54
1.2 "	39.5 "	.54
2.0 "	53 "	.56
5.0 "	73 "	.54
8.0 "	80 "	.50
12.0 "	86 "	.51

These results seem to indicate that the destruction of pepsin under the conditions described takes place in accordance with the law of the bimolecular reaction, and the formula which seems to apply is:

$$\frac{X}{t(1-X)} = K,$$

where X represents the destruction, in hundredths of the original in time t, and K represents a constant.

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Synthesis of uric acid.

By **NELLIS B. FOSTER** and **JAMES C. GREENWAY.**

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons, and the Wards of the New York Hospital.*]

The possibility of a synthesis of uric acid from lactic acid and urea has been considered by a number of investigators, but when these substances are taken by way of the stomach, all researches alike have failed to disclose any evidence of uric acid synthesis in

mammals. The object of this study was first, to repeat the earlier experiments of Minkowski; second, to find out if slight changes of method would perhaps serve to show a synthesis of uric acid; and third, to consider the problem experimentally in relation to a man suffering from chronic gout. In gout it is possible that an abnormal synthesis of uric acid occurs, and also, since the uricolytic powers of the gouty organism are less active than normal, a synthesis masked in a normal person might be evident in a person suffering from gout.

The results of the experiments may be briefly summarized as follows: when lactic acid is administered to a normal man who has been fed on a purin free diet, there is no resulting increase of uric acid in the urine, even when the amounts of lactic acid are very large —*i. e.*, 20 grams in a dose. In a dog on a purin free diet (milk, eggs, and rice), following the hypodermic injection of lactic acid, and of lactic acid and urea, there was in both instances a slight increase in the percentage of total nitrogen excreted as uric acid. The absolute amounts were also slightly increased as were those of allantoin. These figures are difficult to interpret and we are not prepared to assert without further investigation that there is a synthesis of uric acid in the manner described.

In a case of chronic gout the effects of the lactic acid and urea were entirely obscured by the irregularity of nitrogen excretion; periods of nitrogen retention and excretion making it impossible to estimate the effects of the treatment.

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Some critical considerations on the serum diagnosis of syphilis.

By **HIDEYO NOGUCHI.**

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

In its application to the detection of syphilis antibody the Bordet-Gengou phenomenon of complement fixation has received but little consideration in its quantitative aspect. As will presently be pointed out it is only by respecting the quantitative relations of all reagents concerned that the test becomes reliable and delicate. Even with an adequate quantity of antigen, blood cell suspension and the patient's serum the detection of the antibody

by means of complement fixation may or may not be successful according to whether or not appropriate amounts of hemolytic amboceptor and complement are employed. A large excess of either one of these two reagents can prevent the test from revealing the presence of the antibody. While it is easy for a serologist to see why complement should be used in definite and uniform quantity, not every worker seems to be conscious of the disturbing effects which are exerted by an excess of the amboceptor. In view of the overlooking of certain principles of hemolysis by most of the investigators of the present time a brief consideration of this particular subject seems to be advisable. For the sake of convenience I take the example of the antisheep hemolytic amboceptor for illustrating the influence of an excessive amount of the amboceptor upon the phenomenon of complement fixation. The effects exerted by the excessive sensitization is two-fold. The first effect is to augment gradually the activity of guinea-pig's complement by increasing doses of the amboceptor, until a maximum is reached. Thus in the presence of one unit of amboceptor 0.1 c.c. of the complement is usually required to produce complete hemolysis. By using four, eight and twenty units of the amboceptor the same effect is obtainable with $\frac{1}{3}$, $\frac{1}{5}$ and $\frac{1}{10}$ of the 0.1 c.c. of complement respectively. For this reason it is impossible to demonstrate a partial fixation of complement by using more than several units of the amboceptor, and when several units are employed the test suffers in delicacy. The second effect is still more disturbing than the first. It depends upon partial dissociation of the complement from its combination with the antigen and antibody compound. A quantity of syphilis antibody just sufficient to fix 0.1 c.c. of the complement against two units of the amboceptor is no longer efficacious to hold back the complement from partial liberation against the influence brought on by more than four units of the amboceptor. The fixation of the complement by two and three units of syphilis antibody respectively is also quite ineffective to prevent hemolysis when ten and twenty units of the amboceptor are added. Under these conditions the test fails to indicate the presence of any syphilis antibody although it is really present. When eight units of syphilis antibody are employed the fixation of complement becomes so firm that twenty units of the amboceptor can no longer bring about its liberation.

From the foregoing it becomes at once evident that any system of the complement fixation test in which definite and appropriate amounts of these two vitally important reagents are not employed is not delicate and accurate enough to be a reliable diagnostic measure.

Referring to the method of Wassermann I may state that it has all the disadvantages arising from the presence of unknown, but often considerably large amounts of natural antisheep amboceptor contained in human serum. Wassermann was quite unaware that the natural antisheep amboceptor is capable of being reactivated by guinea-pig's complement, and hence he recommended the use of two units of immune antisheep amboceptor of the rabbit with the view of obtaining complete hemolysis. The reactivability of the natural amboceptor by this complement has been discovered since by Bauer who, in turn, proposed to utilize the natural amboceptor and dismiss the use of the immune amboceptor. By systematic examination of more than 100 specimens of human sera in regard to the content of natural antisheep amboceptor I found that it varies from almost none to as many as twenty units in 0.1 c.c., the quantity usually employed for each tube in the fixation test. Thus the method of Wassermann is destined to give unreliable and inaccurate reactions. Bauer's modification is just as inaccurate as the original as it relies upon unknown amounts of the natural amboceptor alone.

The method which I have recently perfected is also a complement fixation test and differs from the Wassermann method in employing an antihuman hemolytic system instead of an antisheep hemolytic system. Thus the human blood corpuscles are to be hemolysed by means of an antihuman amboceptor prepared in the rabbit and the complement of the guinea-pig. I use two units of the amboceptor for each tube. In this new system the danger of introducing any uncalculated amount of the hemolytic amboceptor is absolutely excluded. As the fixation test is carried out with definite and uniform amounts both of the complement and the amboceptor, the results obtained with different specimens and at different occasions are all comparable with one another. The sharpness of the reaction enables one also to follow the fluctuation of the antibody content even to a fraction of one unit.

Just as I had finished my experiments Tschernogubow published an article in which he stated that he successfully employed an antihuman amboceptor in combination with human complement. As he made no statement as to the source and strength of his antihuman amboceptor no judgment can be made on his method. It is rather striking to observe that the amount of the amboceptor he employed was 0.25 c.c. for each tube, in contrast to 0.002 in my method. It may not be entirely out of place to mention here some essential reasons why I use guinea-pig's serum as complement.

According to my observations the amount of complement in human serum varies considerably in different individuals. In the majority of specimens 0.1 to 0.03 c.c. of the fresh serum contain about enough complement to produce complete hemolysis with ten units of the amboceptor, while the same quantities do not cause any marked hemolysis when two units of the amboceptor are employed. Thus ten units of the amboceptor are to be used as a necessary amount for utilizing human serum as complement. Now in regard to the quantity of each specimen of human serum to be used for complement it is essential to determine the exact strength by a preliminary titration, because if we use some excess of complement the test turns out completely negative. It appears probable that human complement, like that of the rabbit, is not very sensitive to the fixing action of the antigen-antibody of syphilis. In this respect guinea-pig's complement is excellent The method which Tschernogubow recommended is to collect a few drops of a patient's blood in saline solution and use the suspension both for the complement and corpuscles at the same time. But, this does not permit one to make any estimation of the complement content of the blood. Moreover, there is no *direct* way of ascertaining whether any inhibition which may be observed with a given specimen is due to the anticomplementary property of antigen alone or to the combined action of the antigen and syphilis antibody, because the complement and antibody exist in the same serum side by side, if this latter is present at all. Again the relying upon human complement makes impossible the testing of any specimen of blood which has been allowed to stand for several days, as the activity of complement rapidly diminishes and

finally disappears under these conditions. His method is not applicable to cerebrospinal fluid.

In contrast to the use of human complement the use of guinea-pig's serum does not possess one of the disadvantages enumerated. The quantity of complement is always uniform and definite. The human complement does not affect the reaction, because the amount present is too trifling to be of any influence. It is possible moreover to employ for the tests an old specimen of blood, dried or moist, by my method, since guinea-pig's complement is used. Unlike Tschernogubow's the present method enables one to repeat the test in case of need.

Before leaving the subject I would like to point out certain advantages from the technical standpoint of the method which I recommend. The quantity of patient's serum[1] required for the test is only two *capillary* drops (one for each of the two tubes) and no preliminary inactivation at 56° C. is necessary. The hemolytic indicator is readily prepared from the patient or a normal person by mixing the blood with physiological salt solution in the ratio of one drop of the blood to 4 c.c. of the saline solution, 1 c.c. of such suspension being used for each tube. The antigen, complement and amboceptor can be used either in liquid or in dried form. This latter, as prepared on filter paper slips, can be preserved permanently under ordinary conditions at room temperature and be employed in place of the corresponding liquid reagents. In this simple form the test should have a wide application to the sero-diagnosis of syphilis and as a measure and control of the efficient treatment of the disease.

37 (375)

On nitrogenous metabolism in chronic nephritis.

By **D. MANSON, L. KRISTELLER** and **P. A. LEVENE.**

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

The present work represents the results of observations on the character of the nitrogenous metabolism in a patient who was

[1] In case of cerebrospinal fluid use 0.2 c.c. for each tube.

placed on a diet containing a sufficient supply of calories and a limited proportion of protein, so that his kidneys could readily remove the end products of catabolism. That the capacity of the kidneys were not overtaxed may be concluded from the fact that the patient remained practically in nitrogenous equilibrium for four months. On this patient it was attempted to establish whether or not the abnormal, as v. Noorden termed it "bizarre" nitrogen elimination observed in course of nephritis is conditioned exclusively by faults of elimination.

CONCLUSIONS.

1. The elimination-capacity of the kidneys of the patient was established by placing the patient on a diet containing a low proportion of protein and a sufficient supply of calories. To this standard diet varying quantities of urea were added, and the rate of the nitrogen output was measured. The output of nitrogen on the standard diet was generally about 5.5 grm. and the addition of 1.5 to 5.0 grm. of nitrogen in form of urea caused a rise in the output never exceeding 6.25 grm. Thus it was concluded that an intake of nitrogen below 7.0 grm. was the most suitable for the condition of our patient.

2. Comparing the rate of elimination of nitrogen after the administration of glycin, alanin, asparagin, with that after administration of urea, one notes a slower rate after the administration of the first two acids, and an equal rate after administration of asparagin (probably owing to the presence of an acid amid group in the molecule).

3. After administration of excessive protein in addition to the standard diet, one notes a much lower rate of nitrogen elimination than one should expect to find in a normal man, on the basis of the work of Falta.

4. Of the total nitrogen removed by our patient in excess over that on the standard diet, 80 per cent. was in form of urea, while in normal man, as calculated from the tables of Folin, the proportion of urea varies between 90 and 100 per cent.; in a normal dog the proportion is always 100 per cent.

5. On the basis of these observations it was concluded that in our patient the rate of conversion of protein into simple nitrogenous substances and into urea is below the normal.

6. The patient remained for four months in a condition of nitrogenous equilibrium, and otherwise in good health, on a diet containing about 6.5 grm. of nitrogen and 3,000 calories, which were ultimately reduced to 2,500 calories to prevent a constant gain in weight.

38 (376)

The formation of gluconic acid by the olive-tubercle organism and the function of oxidation in some microörganisms.

By **CARL L. ALSBERG.**

[*From the Office of Poisonous Plant Investigation, Bureau of Plant Industry, U. S. Department of Agriculture.*]

The olive-tubercle organism, *Bacterium savastanoi*, recently described by Erwin F. Smith,[1] when grown in the presence of glucose and an excess of calcium carbonate, converts the greater part of the glucose into calcium gluconate. The amount of energy liberated thereby is exceedingly great in comparison to the weight of the organisms. This is to be explained by the fact that the energy requirements of microörganisms are very much greater than those of higher forms, partly because of the disproportion between the body surface and the body volume of microorganisms, and partly because microörganisms exist in a medium which is an excellent conductor of heat.

39 (377)

On the fertilizing and cytolytic effect of soap.

By **JACQUES LOEB.**

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

It has been shown by experiments on the eggs of sea-urchins, starfish, and annelids that the artificial membrane formation is the act which causes the unfertilized egg to develop. The agencies which cause the artificial membrane formation, as a rule, injure the egg. For the eggs of the starfish and certain other annelids

[1] Erwin F. Smith: Recent Studies of the Olive-Tubercle Organism. U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin No. 131, Part IV, Washington, 1908.

this injury is rather slight, and these eggs are able to develop into larvæ without any further treatment. In the egg of the Californian sea-urchin this secondary, injurious effect connected with the artificial membrane formation is more severe and demands a further treatment of the egg. This consists in preventing the eggs from developing for about from two to three hours after the membrane formation, by depriving them of oxygen or by preventing oxidations in the egg through the addition of a trace of potassium cyanide. During this time the egg is able to recuperate from the injurious effects of the membrane formation and is able to develop perfectly normally into a pluteus if transferred into normal sea-water. In my first experiments with this method, four years ago. not more than ten per cent. of the eggs could be caused to develop in this way. I have recently found that by a slight improvement of the method all the eggs can be caused to develop into larvæ, The segmentation is as a rule as normal as if the eggs were fertilized by sperm. A second method of overcoming the injurious effect caused by the artificial membrane formation consists in putting the eggs for from 10 to 40 minutes into hypertonic sea-water. This method also causes all the eggs to develop.

These experiments showed that the process of membrane formation is the real cause which starts the development of the unfertilized egg; and the question therefore arose what the nature of this process is. My recent experiments have shown that the agencies which cause hemolysis also cause the membrane formation and the development of the unfertilized egg. I have thus been able to show that saponin, solanin, digitalin, bile salts, fatty acids, alkalis, hydrocarbons, ether and alcohols and the blood serum of not too closely related forms cause the membrane formation of the unfertilized egg and its subsequent development. There remained only one cytolytic substance which seemed to form an exception, namely, soap, but experiments which I have recently carried out have shown that it is possible to cause the membrane formation and subsequent development of the egg with sodium oleate.

If the unfertilized eggs of the sea-urchin are put into a mixture of 50 c.c. N/2 sodium chloride + 0.2 c.c. N/10 sodium oleate, the eggs form no membrane, nor do they undergo cytolysis; but

if they are transferred into sea-water they form membranes and a smaller percentage of them undergoes cytolysis. If the eggs remain a short time only in the soap solution, they all form membranes, but few cytolyze after being transferred into sea-water; if they remain for a longer time, they all form membranes but cytolysis follows very soon after the membrane formation.

The question arises, why do the eggs form their membrane only after they are transferred into sea-water? This is due to the alkaline reaction of the sea-water. If we make the sea-water faintly acid by the addition of hydrochloric acid no egg forms a membrane or undergoes cytolysis after being transferred into sea-water, and if we make the solution of sodium oleate in sodium chloride slightly alkaline by the addition of sodium hydroxide the eggs form membranes while they are in the soap solution.

If we allow the soap solution to act only long enough to cause the membrane formation, but not long enough to cause cytolysis, the eggs can be caused to develop larvæ. We may from all these experiments draw the inference that the development of the resting egg is caused by a superficial or mild cytolysis, and that the spermatozoon must carry a cytolytic substance into the egg, possibly a trace of higher fatty acid.

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On the depression of the freezing point of water due to dissolved caseinates.

By **T. BRAILSFORD ROBERTSON** and **THEODORE C. BURNETT.**

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

The question whether or not proteins possess, in solution, a definite osmotic pressure has been the subject of much controversy. The original investigations of Graham[1] appeared to indicate that colloids in general exert a high osmotic pressure. Subsequent investigators, however, attribute these results to an admixture of crystalloids and the investigations of Sebanejew,[2] Tamman,[3]

[1] Graham: *Phil. Trans. Roy. Soc.*, 1861, cli, 183.

[2] Sebanejew: *Berichte d. deut. chem. Gesell.*, 1890, xxiii, 87; 1891, xxiv, 558; xxvi, 385. Sebanejew and Alexandrow: *Journ. of the Russian Phys.-chem. Soc.*, 1891, p. 7; quoted after *Maly's Jahresber. f. Tierchem.*, 1891, xxi, 11.

[3] Tamman: *Zeit. f. physikal. Chem.*, 1896, xxi, 180.

Dreser,[1] Koeppe[2] and others indicate that when they are carefully freed from associated inorganic substances the cryoscopic depression due to dissolved proteins is negligible, while Reid[3] finds that proteins purified by repeated recrystallization, resolution and recrystallization frequently possess, in solution, no measurable osmotic pressure ; and he concludes that provided every precaution be taken to exclude impurities (among which he includes inorganic constituents) from the protein solution it will be invariably found to possess no osmotic pressure whatever and that the osmotic pressures observed in solutions incompletely purified are due, not to protein, but to the associated impurities.

It appears to us that many of the above-quoted observations and conclusions are vitiated by the fundamentally erroneous conception that the inorganic constituents which are found associated with proteins are invariably present as impurities and not in a state of chemical combination. The manner in which this assumption vitiates conclusions regarding the molecular weight (estimated from the depression of the freezing-point or directly from the osmotic pressure) of proteins will be clear from the following considerations: Bases and acids have been demonstrated to form definite salts of a constant composition with casein, serum globulin and protamin, and there can be no doubt whatever that similar compounds are formed with other proteins. In solutions of casein and of serum globulin it can be shown that as the neutral point is approached the alkali-binding power becomes less and from a variety of data it can be shown that this phenomenon is due to a polymerization of the protein molecule according to equations of the type: $HXOH + HXOH = HXXOH + H_2O$[4] so that at or in the neighborhood of the neutral point molecular aggregates are formed of such dimensions that, in the cases of the proteins mentioned, the solution assumes the character of a suspension and the protein is precipitated; addition of acid or alkali shifts the equilibrium in the direction of the lower complexes and the protein goes into solution again in the form of a salt. Similar phenomena may be safely as-

[1] Dreser: *Arch. f. exper. Path. und Pharm.*, 1892, xxix, 314.
[2] Koeppe: *Arch. f. d. ges. Physiol.*, 1896, lxii, 571.
[3] Reid: *Journ. of Physiol.*, 1904, xxxi, 438.
[4] T. Brailsford Robertson: *Journ. of Physical Chem.*, 1908, xii, 473.

sumed to occur in other protein solutions, although the polymerization of the protein, which occurs when the uncombined protein is set free, may not result in actual precipitation. The elaborate precautions which have been taken by many observers to free the protein under investigation from accompanying inorganic substances, have, therefore, defeated their own ends by converting the protein into molecular aggregates so enormous as to possess a necessarily immeasurably small osmotic pressure.

Since it appears probable, therefore, that the dissolved *salts* of proteins may exert a measurable osmotic pressure in solution, and hence, cause an appreciable lowering of the freezing-point of water in which they are dissolved, we have undertaken a series of determinations of the lowering of the freezing-point of water, which is brought about by dissolved (neutral) caseinates.

The solutions are made up as follows: Alkali of a given concentration is shaken up with excess of casein until no more casein will dissolve and the solution is then filtered through rapid-filtering paper. The resulting solution is a solution of the "neutral caseinate" of the base and is neutral to litmus.[1] The cryoscopic depression is estimated in the usual way. The following are the results which have so far been obtained:

Experimental error of determination $\pm$ 0.0025°.

Base.	Concentration of base "saturated" with casein.	Δ	Indicating a concentration of
NH_4OH	$m/50$	0.045	$m/41$
"	$m/33.3$	.055	$m/33.6$
KOH	$m/50$	.0325	$m/57$
"	"	.0375	$m/49.3$
"	$m/33.3$	.0425	$m/43.7$
"	"	.0475	$m/38.9$
"	$m/20$	.05	$m/37$
"	"	.075	$m/24.6$
"	$m/15$	.1	$m/18.5$
LiOH	$m/59.5$	.03	$m/61.6$
"	$m/39.6$	.045	$m/41$
"	$m/23.8$	.07	$m/26.4$
"	$m/17.8$	.08	$m/20.3$

Since these solutions are neutral and no inorganic substance is introduced save the base employed to dissolve the casein it is evident that the compounds of bases with casein cause, in solution in

[1] T. Brailsford Robertson: *Journ. of Biol. Chem.*, 1907, ii, 336.

water, a definite cryoscopic depression. In harmony with deductions from titration- and conductivity-data[1] the results are such as indicate that casein behaves towards bases, essentially as a monobasic acid possessing a molecular weight, in solutions neutral to litmus, of approximately 2,000.

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The daily excretion of bacteria in the feces of healthy men.

By **W. J. MACNEAL, LENORE L. LATZER** and **JOSEPHINE E. KERR.**

[*From the Laboratory of Physiological Chemistry, Department of Animal Husbandry, University of Illinois, Urbana, Ill.*]

During the past year we have examined at intervals of about two weeks the fecal bacteria of each individual in a group of twelve men who were the subjects of a prolonged metabolism experiment. These men were fed a mixed diet, in quantity according to their choice at the beginning of the experiment. Altogether we have examined bacteriologically 266 stools.

The quantity of bacteria in each of these stools was estimated by two different methods of microscopic counting and in about half of them the quantity of bacterial dry substance was also estimated by the gravimetric method of Strasburger.

In the individual examinations the largest number of bacteria observed was 816×10^9 bacterial cells per gram fresh feces, $2{,}642 \times 10^9$ bacterial cells per gram dry feces. The smallest number of bacteria counted was 124×10^9 per gram fresh feces, 983×10^9 per gram dry feces. By the gravimetric method the largest quantity of bacterial dry substance observed was 42.53 per cent. of the fecal dry substance or 13.2 per cent. of the moist feces. The smallest quantity of bacterial dry substance observed was 14.03 per cent. of the fecal dry substance or 2.6 per cent. of the moist feces. The average of all examinations was 375×10^9 bacterial cells per gram fresh feces; $1{,}587 \times 10^9$ bacterial cells per gram dry feces; bacterial dry substance in fecal dry substance,

[1] T. Brailsford Robertson: *Journ. of Physical Chem.*, 1908, xii, 479, etc.

26.89 per cent.; bacterial dry substance in moist feces, 7.0 per cent. The average daily quantity of fecal dry substance and of fecal nitrogen was calculated from the analysis of eight-day period collections, and the average daily bacteria and bacterial nitrogen calculated upon these quantities, regarding the bacterial content of the dry substance of the single stool, upon which the bacteria were determined, as representative of the feces for that period. Calculated in this way the greatest values found were 58×10^{12} bacterial cells per day by count, and 9.15 grams bacterial dry substance, containing 1.006 grams nitrogen, per day by the gravimetric method. The smallest values were 14×10^{12} bacterial cells and 1.87 grams bacterial dry substance, containing 0.194 gram nitrogen per day. The average of all examinations was 32×10^{12} bacterial cells, and 5.34 grams bacterial dry substance, containing 0.585 grams of nitrogen, per day.

There was considerable individual variation among the different subjects of the group. For example, the average of all examinations of Subject H was 40×10^{12} bacterial cells and 7.26 grams dry bacterial substance, containing 0.819 gram nitrogen, per day. The average of all examinations of Subject B was 26×10^{12} bacterial cells and 3.56 grams bacterial dry substance, containing 0.393 grams nitrogen, per day.

The nitrogen contained in the bacteria varied from 66.8 per cent. to 23.3 per cent. of the total fecal nitrogen, the average of all examinations being 46.3 per cent. Individual variation in this respect was also considerable. In Subject I the average of all examinations showed 57.4 per cent. of the total fecal nitrogen contained in the bacteria; in Subject K this quantity was 32.1 per cent. These two subjects were the ones who showed the two extremes (66.8 per cent. and 23.3 per cent., above) for the single examinations.

A detailed account of these experiments will be published in a short time in *The Journal of Infectious Diseases.*

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Further studies on the constitution of inosinic acid.

By **WALTER A. JACOBS** and **P. A. LEVENE.**

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

In a former paper[1] we have already communicated that the inosin which we obtained from inosinic acid was identical with that obtained from karnin by Haiser and Wenzel.[2] We have now succeeded in isolating from inosin the pentose in a crystalline state. The properties of this sugar are as follows: Melting point 87° C. Its rotation in aqueous solution is $(d)_D = -19°.4$. The osazone melts at 163°–164° C. and shows a rotation when 0.2 gram are dissolved in 10 c.c. of a mixture of four parts pyridine to six parts of alcohol of $(d)_D = -0°.92$. The benzylphenylhydrazone melts at 128° C. and in absolute alcoholic solution rotates $(d)_D = -26°.46$.

We therefore conclude that this sugar is neither xylose nor arabinose as stated by Neuberg and Brahm[3] and Bauer[4] respectively. We hope, by further study, to establish its exact nature.

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The effect of heat on the anaphylactic properties of proteins.

By **JOHN F. ANDERSON** and **M. J. ROSENAU.**

[*From the Hygienic Laboratory, P. H. and M. H. S., Washington, D. C.*]

We have demonstrated that horse serum, egg-white and milk when dried, then heated and redissolved, possess unaltered powers of sensitizing and poisoning guinea-pigs in the sense of hypersusceptibility.

The above named substances, when thoroughly dried, were heated to 130° C. for two hours, 150° C. for ten minutes, or 170° C. for ten minutes. We have previously shown that both the sensitizing and toxic properties of liquid horse serum are gradually in-

[1] PROC. SOC. EXP. MED. AND BIOL., 1909, vi, 56. *Ber. d. deutschen chem. Gesell.*, 1909, xlii, 335.

[2] *Monatshefte für Chemie*, 1909, xxix, 157.

[3] *Ber. d. deutschen chem. Gesell.*, 1908, xli, 3376.

[4] *Beiträge zur chem. Physiol. und Path.*, 1908, x, 345.

fluenced by heat and are practically destroyed at about 100° C. The difference probably depends upon coagulation of the protein and consequent failure of absorption.

Dried sensitive guinea-pig blood serum, containing anaphylactin, withstands at least 100° C. for ten minutes.

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A skin reaction in carcinoma from the subcutaneous injection of human red blood cells.

By **CHARLES A. ELSBERG.**

[*From the Mount Sinai Hospital.*]

Numerous investigators have shown that if the blood serum of a patient suffering from carcinoma be mixed with normal human red blood cells hemolysis occurs. The reaction takes place in from 50 per cent. to 80 per cent. of patients with malignant disease. It occurs with considerable frequency in tuberculosis, and more rarely in other diseases.

It occurred to the writer, that, by the injection of red blood cells under the skin of the carcinoma patient, it might be possible to produce a local reaction at the site of the injection. Logically, a local hemolysis should take place. Theoretically, such a reaction might prove to be a delicate one ; it might give more positive and definite results than the test-tube method. In the technique which is used for the test-tube method the presence and degree of hemolysis is indicated by the amount of laking of the red cells — that is, by the amount of hemoglobin which has been set free. The tube reaction gives no evidence of other substances than the hemoglobin which have been liberated. A small amount of hemolysin in the serum which is being tested might not be capable of detection. If normal human blood cells are injected under the skin of a patient whose serum is hemolytic, fresh quantities of hemolysin would be continually carried to the cells, and therefore even a small amount of hemolysin might cause hemolysis of the cells. Every organic substance which was set free would enter the tissues and might there have its effect.

Accordingly, after some experimentation, normal blood was in-

jected under the skin of patients suffering from carcinoma or other disease. In patients with malignant disease, a decided local reaction was observed. The technique employed was a very simple one. Under aseptic precautions, blood was aspirated from the median basilic vein of a normal individual (preferably a child), every possible precaution being taken that the individual was healthy and free from hereditary or acquired disease. The blood was defibrinated, and the cells washed four times in normal saline solution, care being taken that the washings and centrifuging was thoroughly done. A 20 per cent. emulsion of the red cells in normal saline solution was made and kept in the ice-box for 24–48 hours before it was used. Five minims of this 24–48 hour old suspension of washed red blood cells were subcutaneously injected into the anterior surface of the forearm of the patient. In the patients in whom a "reaction" was obtained, the following changes were noted in the skin at the site of the injection. Six to eighteen hours after the injection, the affected area was slightly raised and slightly tender, it had a more or less well defined margin, it measured from two to four centimeters and it was of a somewhat dusky red color. The changes in the skin reached their maximum within one or two hours, and the red area then began to fade, rapidly or slowly. Eight to twenty four hours after the injection, the skin lesion had either entirely disappeared, or more often, a brownish, bluish or lemon-yellow discoloration remained, which persisted for a number of days.

In the patients who did not show this reaction, there was either nothing to be seen at the site of the injection excepting the needle puncture, or a brownish discoloration of the skin, or a bluish discoloration, as is often seen after a hypodermic injection.

My investigations of this cutaneous lesion are very incomplete. I have given thirty four injections to twenty patients with known carcinoma, and every one of the cases had a positive reaction. In most of the patients several injections were given of different blood cells. With succeeding injections, the reaction was either less marked or failed to appear. Of four patients with known sarcoma, three gave a positive reaction. Injections were given to over one hundred normal individuals or to those suffering from diseases other than sarcoma or carcinoma. These included a

variety of diseases, such as nephritis, tuberculosis of lungs, bladder, kidneys (7 cases), leukemia, syphilis (2 cases), benign tumors, acute and chronic inflammatory affections, etc. In all but three cases, the reaction was negative. One patient with a septic endocarditis gave a suspicious, but not a positive reaction. Two other injections made in this patient were negative. This case was among the earliest of our series, and therefore must be accepted with caution. A patient who had been operated on for a large rapidly growing lymphangioma of the suprapubic region and had several large angiomata of the thigh with a large post-operative hematoma of the scrotum, gave a marked positive reaction. A patient with gastric symptoms, absence of hydrochloric acid and presence of lactic acid, with a loss of thirty pounds in weight, gave a positive reaction. The operation failed to show any carcinoma. I obtained no reaction in several patients in whom malignant disease was suspected but in whom no malignant disease was found at operation or at autopsy, and have obtained positive reactions in several patients in whom carcinoma was not suspected, but in whom carcinomatous disease was found at operation. Jaundice seemed to have no influence upon the appearance of the reaction.

In order to gain a more definite idea of the causation of the skin changes, normal defibrinated blood was laked with distilled water, and, after the tubes had been centrifuged, the supernatant fluid was injected into a number of patients. For purposes of control, hypodermic injections of sterile distilled water were given. All of the patients, whether suffering from malignant disease or not, who received hypodermic injections of laked blood, presented a skin lesion similar to that which was observed in carcinoma patients after the injection of washed red blood cells. This seemed to show that in the patients with carcinoma the reaction from the blood cell injection was due to a local hemolysis. In the normal individual or one suffering from some other disease, on the other hand, there was no hemolysis and therefore no reaction. When, however, the hemolyzable substances extracted from the red cells by laking were injected, the characteristic local lesion was observed. I have not yet been able to obtain pure human hemoglobin for injection purposes.

A fuller account of the technique employed and a more exact description of the cutaneous lesions observed, of the possible value of red blood cell mixtures, *i. e.*, of suspensions of the mixed red blood cells of several individuals, of the possible value of some animal red blood cells for injection purposes, of the significance of the persistence or disappearance of the reaction after an operation, and many other aspects of the subject will be discussed in a future communication. The number of injections thus far given is far too small to allow of positive conclusions as to the reliability of this method for diagnostic purposes. The results obtained thus far have been striking. The purpose of this preliminary report is to call attention to the fact that it is possible to cause a local hemolysis in the living body by the subcutaneous injection of washed normal human red blood cells, and that in patients with malignant disease, especially carcinoma, a characteristic and easily recognizable local skin lesion is caused by this injection.

Thirty third meeting.

Cornell University Medical College, New York City.
April 21, 1909. President Lee in the chair.

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The vascularity of the spleen as influenced by single nerves of the plexus lienalis.

By **R. Burton Opitz.**

[*From the Physiological Laboratory of Columbia University.*]

The blood supply of the spleen was determined by means of a stromuhr inserted into the splenic vein. The average for a dog of 18.0 kilos body weight, with a spleen weighing 100 grm., amounted to 0.97 c.c. per second, the velocity of the blood stream to 42.0 mm. per second, the venous pressure to 10.0 mm. Hg.

Vaso-constrictory influences were obtained on stimulation of either splanchnicus major, or of the plexus lienalis. Single nerves of the plexus, designated as α, β, γ and δ also showed strong vaso-constrictory powers. Thus, it was possible to transfer from 30 to 50 c.c. of blood from the spleen into the systemic circuit by moderate stimulation of either one of the nerves just mentioned. The removal of so large a quantity of blood from this organ by the constriction following the stimulation, resulted in a rise in general blood pressure.

On the venous side the vaso-constrictions made themselves felt by:

1. A quick sharp rise in the venous return and venous pressure.
2. A gradual decrease in the blood-flow and pressure.
3. A slow adjustment toward normal values.

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An experimental study of the influence of kidney extracts and of the serum of animals with renal lesions upon the blood pressure.

By **RICHARD M. PEARCE, M.D.**

[*From the Carnegie Laboratory of the University and Bellevue Hospital Medical College, New York City.*]

1. Extracts of the rabbit's kidney injected into the rabbit cause a slight increase in blood pressure which is barely more than that due to the mechanical effect of the injection.

2. Extracts of the dog's kidney injected into the dog cause a decided fall in pressure; an equal fall may be caused by the dog's urine. A series of control experiments indicates that the fall caused by the kidney extract may be due to the urinary salts which it contains.

3. Extracts of cat's kidney cause a rise in pressure; as the cat's urine causes a fall, this rise in pressure indicates the possibility of a kidney extract containing a pressor substance which cannot be influenced by the depressor substance of the urine.

4. Rabbit's kidney which in the rabbit produces a slight rise when injected into the dog causes a drop comparable to that caused by the dog's kidney itself. Similarly the dog's kidney, which injected into the dog causes a drop, produces in the rabbit a rise analogous to that produced by rabbit's kidney. It is evident therefore that these pressor and depressor substances of the kidneys in question do not have a constant effect on all animals as do the extracts of the adrenal gland.

5. Extracts of kidneys which are the seat of various forms of nephritis cause the same effect as extracts of normal kidneys.

6. The serum of dogs with considerable reduction of kidney substance causes a slight fall in pressure; the serum of dogs with spontaneous nephritis gives divergent results, as does also the serum of rabbits with various forms of acute nephritis. The serum of dogs with chromate nephritis causes a slight rise, while that of dogs with uranium nephritis produces a sharp and decided fall in pressure. Although there is no uniformity in these results, their

general character, and especially the experience with uranium and chromate sera of the dog, suggests that pressure-disturbing substances are present in the serum as the result of the kidney lesion. The very slight evidence of the constant presence of a pressor substance, however, offers little support to the theory that such a substance is furnished by the diseased kidney or is due to disturbances of metabolism caused by disease of the kidney.

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Further observations on the effect of asphyxia and curare on the reducing power of the blood after section of the hepatic nerves in dogs.

By **J. J. R. MACLEOD.**

[*From the Laboratory of Physiology, Western Reserve University, Cleveland.*]

In a previous communication on this subject (Macleod, *American Jour. of Phys.*, 1909, XXIII, 278) it was concluded that section of the hepatic nerves does not prevent the establishment of hyperglycæmia as a result of asphyxia and curare poisoning (Table III, p. 293, *loc. cit.*). The conclusion was based on the results of three experiments in which asphyxia was practiced, and in two of which marked hyperglycemia was observed; and on one in which curare was injected. Subsequent experiments of the same nature have yielded results which do not corroborate the above conclusion for asphyxiated animals, but do so for those which are curarized.

The following table gives the results of these experiments:

No. and nature of experiments.	*Per cent. of reducing substance in blood.*	
	Before.	*After.*
102 asphyxia	0.104	0.121 (30 min.). 0.147 (90 min.).
104 asphyxia	0.244 0.244	0.262 (60 min.).
105 asphyxia	0.200 0.214	0.176 (45 mm.).
107 asphyxia	0.113	0.155 (45 min.). 0.138 (75 min.).
108 curare	0.265	0.304 (30 min.). 0.363 (45 min.). 0.354 (75 min.).
109 curare	0.178	0.334 (40 min.).
110 curare	0.146	0.225 (60 min.). 0.272 (90 min.).

48 (386)

Toxin-antitoxin mixtures as immunizing agents.

By **WILLIAM H. PARK** and **EUGENE FAMULENER.**

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

Ehrlich early suggested that injections of partially neutralized diphtheria toxin produced active immunity. This was demonstrated by Wernicke, Dreyer and Madsen, Morgenroth, ourselves and others. Smith in a recent article suggested the use of such mixtures in the immunization of children. The possibility of such a practical application has suggested to us some experiments with especial reference to the safety and effectiveness of the injections.

The proportion of toxin to antitoxin in the mixture required to produce immunization. — In May, 1903, one of us reported some experiments in which one set of horses were injected with mixtures containing toxin .66 of L+ dose for each unit of antitoxin and another with .66 of L+ for each 4 units of antitoxin. Three large injections produced in the first series an average of 150 units, in the second an average of 3 units in each c.c. of serum.

Smith injected three guinea pigs with 1 unit plus 1 L+ dose, 2 units plus 1.3 L+ dose, and 2 units plus 1 L+ dose respectively. The litters born from the first and second animals showed marked immunity at the end of eight months. The litter from the third animal showed slight immunity at three months and none at six months.

These experiments indicate that while even a proportion of 6 units of antitoxin to 1 L+ of toxin produces slight immunity, the toxin must be in proportion of more than 1 L+ dose to 2 units to give marked and lasting effects.

This brings us to the question of the safety of such mixtures. The work of Morgenroth suggests that mixtures which are toxic for one species are toxic for all. There is a difference, however, among animals as to the amount of natural immunity and perhaps therefore as to the development of serious symptoms or death from a given quantity of toxin-antitoxin mixture. Even if all guinea pigs lived, therefore, there would be a slight uncertainty in infants.

In a series of tests we found that the least proportion of diphtheria toxin which was necessary to give lasting immunity was not quite harmless in guinea pigs. Thus of four guinea pigs receiving a mixture of 1 unit plus $\frac{1}{2}$ L+ the two larger remained permanently well, while the two smaller finally died of paralysis. Four guinea pigs receiving one half the quantity of the same mixture all remained alive. Two other series receiving still larger quantities of the same mixture acted as the first lot. Some of these guinea pigs received six months later two fatal doses of toxin without serious poisoning.

It is interesting to note that three of these animals received repeated injections of toxin in increasing amounts, until finally 6,000 fatal doses were given in one injection. The blood of the animals at this time contained from 25 to 30 antitoxin units per cubic centimeter. It is possible that the toxin used in these experiments which was produced by Culture No. 8 may have more tendency to promote late paralysis than that from other cultures such as used by Smith.

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Antiperistalsis in its relation to tubercle bacilli and other bacteria in the alimentary tract.

By **ALFRED F. HESS.**

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

Fifteen years ago Grützner showed that charcoal, starch and similar substances when introduced in normal salt solution into the rectum, ascended in the intestinal tract and after from four to six hours could be demonstrated in the stomach. His work was confirmed by some and refuted by others. This problem has of late assumed a new aspect inasmuch as some workers have claimed that when bacteria are introduced in the same way, within a short time they ascend by means of the antiperistaltic action of the alimentary tract to the stomach, œsophagus and thence into the respiratory tract. The most recent report of this phenomenon comes from the Kaiserliche Gesundheitsant, which concludes that this antiperistaltic movement must be considered, not only in exper-

imental studies on tuberculosis, but also in the prophylaxis against infectious diseases; more especially in the disinfection in cases of cholera and typhoid fever, where the sputum may in this way be contaminated.

The experimental facts claimed are: When *Bacillus prodigiosus*, tubercle bacilli or other bacteria are suspended in from 10 to 20 c.c. of salt solution and are injected by rectum into an adult rabbit, they may be recovered by culture or animal inoculation an hour later from the small intestine, stomach, œsophagus, trachea, lungs and other organs. These results are interpreted as proving conclusively that these bacteria, after traversing the gastro-intestinal tract, have entered the trachea and lungs, and thence have been transported throughout the body.

The technique previously employed was, in brief, to keep the animal securely fastened following the injection, so that he could not lick himself, to kill him after a variable period, and then under the strictest asepsis to make numerous cultures from the contents of the alimentary tract, from the macerated organs, and from the heart's blood.

It appeared that the foregoing facts could, for the most part, be conceded, and nevertheless be open to quite a different interpretation. With this question in view I undertook the following series of experiments.

The technique which I made use of differs in two respects from that used by others: (*a*) Large amounts of blood obtained for examination were drawn from the ear vein or jugular vein, previous to pithing the animal; (*b*) care was taken to avoid contact with blood in the cultures made from the hollow viscera. In addition it was found necessary to introduce other experiments.

The following is a summary of my results:

Of four experiments in which the animals were killed within three hours of the rectal injection, in three bacteria were not present above the ileo-cæcal valve; in one they were obtained in the small intestine after seventy minutes; in no instance could they be cultivated from the stomach or œsophagus.

Furthermore of four other experiments where twenty-four hours was allowed to elapse before killing the animal, in one the tubercle bacillus was demonstrated by means of inoculation to be

present in the stomach. In the three others the injected bacteria were not found in the alimentary tract.

In the various experiments bacilli were recovered from the lungs, liver and kidneys, urine and mesenteric glands.

In three of four experiments *Bacillus prodigiosus* was cultivated from the blood within three hours of the rectal injection, and once twenty-four hours later. In two instances, where attempt was made tubercle bacilli were found in the blood twenty-four hours after they had been introduced into the rectum. Forty-five and fifty milligrams of a bovine culture had been injected, and 2 or 3 c.c. of blood was inoculated into each of six guinea pigs in both instances. In none of these experiments was the organism found in every blood culture, and in many of them the heart's blood tested proved to be sterile.

The result of these blood cultures suggested the injection of the bacilli directly into the circulation with an injury as to their subsequent distribution in the body. This work is still unfinished, but it may be of interest to note in this connection that one hour after injecting one forty-eight hour agar culture of *Bacillus prodigiosus*, this organism was found in the small intestine, and that three hours following the injection of 130 mg. of tubercle bacilli they were demonstrated in the stomach and small intestine. That these tubercle bacilli did not enter the alimentary tract by way of the lungs was shown by two experiments in which the pylorus was ligated previous to the intravenous inoculation and the bacteria were found in the small intestine. In these instances the bacteria either passed into the lumen of the stomach or intestine from without, or entered by means of the bile passages. Further experiments are being carried out to determine this question.

After it was shown that the bacteria entered the blood following introduction into the rectum, it seemed necessary to inquire whether they were excreted by the salivary glands and could possibly in this way enter the upper part of the alimentary tract. To this end three experiments were carried out on dogs. Rectal injections of *Bacillus prodigiosus* were given in the manner described and two or three hours later the secretion from the parotid gland was obtained by means of a capillary tube inserted in the opening of Stenson's duct. In most instances this is easy to carry

out and five to ten cubic centimeters of saliva can thus be obtained after giving the dog 1/100 gr. of pilocarpine subcutaneously. None of the cultures made from the saliva showed *Bacillus prodigiosus;* however, I do not believe that this route is absolutely excluded.

One clinical test was made. A "typhoid-carrier" who is known to have had typhoid bacilli in her stools for some years, at times almost in pure culture, but at present in the ratio of about ten per cent. of the total number of fæcal bacteria, was submitted to an examination. If the bacteria ascend from the intestine then typhoid bacilli should be found in her stomach. This patient was starved for eight hours; at the end of the period her mouth was washed with sterile salt solution, and the washings tested for typhoid bacilli. Her stomach, which was found to be empty after this period, was accordingly washed out and the washings, which were of neutral reaction, likewise plated on Conradi-Drigalski media. Neither of these fluids was found to contain typhoid bacilli, nor indeed colon bacilli.

From these experiments I conclude that bacteria injected by way of the rectum into rabbits are not carried in a viable state above the small intestine, and that they do not enter the respiratory tract by this route. In fact their presence in the small intestine may at times be due not to antiperistalsis, but to excretion from the blood or the bile. Furthermore; where experiment has showed them to be present in the lungs, the trachea and the œsophagus, they have entered these organs by way of the blood stream.

50 (388)

The action of soaps on the pneumococcus.

By **SIMON FLEXNER** and **RICHARD V. LAMAR.**

[*From the Rockefeller Institute for Medical Research, New York.*]

The object of the study to be reported briefly is the ascertaining of the manner in which the pneumococcus is disposed of in the body of infected animals that recover. The animal experiments were made on full grown rats. A strain of pneumococcus fatal to them in 1/10,000 of a cubic centimeter of a twenty-four hour bouillon culture was employed. Strong solutions (1 to 5 per cent.)

of soap (sodium oleate) precipitate the diplococci in an adherent mass which afterwards undergoes complete solution in water or salt solution. Solutions of soap of a strength of 1 to 10,000 do not produce visible changes in the bacterial suspensions but reduce slightly the number of viable cocci. Solutions of 1 to 15,000 or 1 to 20,000 do not affect the viability in cultures but reduce somewhat the virulence. At the same time the diplococci appear somewhat swollen but not otherwise altered.

Untreated diplococci begin to multiply at once in the peritoneal cavity of rats. The treated diplococci at first almost entirely disappear from the cavity and begin to multiply after eight or more hours and cause death at a later period than the controls. There is a greater emigration of leucocytes in the case of the treated cocci than in that of the controls. There is little or no phagocytosis. Normal goat serum does not affect the process appreciably; but immune goat serum prevents multiplication of the treated cocci and brings about recovery of the rats but, under the conditions of the experiment, not of the control rats injected with untreated cocci. Phagocytosis does not play a direct part in the recovery.

The experiments can be repeated *in vitro* with approximately similar results. The soap-treated cocci are subject to serum lysis, while the untreated are not, and the lysis is not assisted but rather hindered by the presence of living leucocytes. The study is being continued.

51 (389)

The influence of shaking upon trypsin and rennin and a comparison of this influence with that upon pepsin.

By **A. O. SHAKLEE** and **S. J. MELTZER.**

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

At the December meeting of this Society we mentioned our studies of the effects of shaking upon ferments and reported that pepsin can be practically destroyed by shaking. Our studies were extended to other digestive ferments and we wish now to report very briefly that shaking proves to be very injurious also to trypsin

and to rennin. Trypsin was tested by the casein method of Gross and the rennin was determined by the method of Blum and Fuld with slight modifications which will not be discussed here. Both ferments were shaken at room temperature, and at 33°C.; also the influence upon the results of different rates of shaking and of changes in other conditions were investigated. We shall, however, state here only that the destructive effect of shaking upon trypsin and rennin is, as for pepsin, distinctly increased by increasing the rate of shaking and by increasing the temperature at which the shaking is carried on. There is a pronounced difference in the resistance to shaking between pepsin and trypsin under the conditions thus far studied, the latter being more readily affected. The destructibility of rennin runs practically parallel with that of pepsin.

52 (390)

The influence of sodium and calcium upon direct and indirect muscle irritability and their mutual antagonistic actions.

By **DON R. JOSEPH** and **S. J. MELTZER.**

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

By the researches of Kühne, Biedermann, Ringer, Loeb, and many others, it is established that solutions of sodium chloride cause rhythmical movements of the muscles of the frog, and that the addition of a small quantity of calcium will stop them. By the researches of Locke, Carslaw, Cushing, Poljakoff and Overton, it is further established that solutions of sodium chloride abolish indirect irritability, and that the addition of a small dose of calcium restores it. There has been very little work done on the *primary* effect of calcium upon the direct and indirect irritability of the skeletal muscles of the frog and there are practically no researches on the action of sodium upon the primary effects of calcium.

In our experiments, sodium and calcium chlorides were employed in M/10 solutions and were introduced by infusion through the abdominal aorta according to the method described by Cushing.[1] The graphic records were obtained from the gastro-

[1] Cushing: *American Jour. of Physiol.*, 1902, vi, 77.

cnemius and the sciatic plexus was stimulated at about one minute intervals by two consecutive shocks (make and break) from an induction current.

In agreement with the above mentioned statements, we found that sodium chloride reduces indirect (curare-like action) and reduces moderately also direct (Poljakoff) irritability. Both are promptly restored by the addition of a small dose of calcium. As a new fact we may mention that the irritability is more readily abolished in cooled frogs.

Although calcium *restores* indirect irritability when abolished by sodium, it *abolishes* indirect irritability when injected primarily. The dose necessary is considerably smaller than that of sodium for the same effect. Again, the indirect irritability thus abolished by primary infusion of calcium can be restored by sodium of which, however, a larger dose is required than of calcium in a secondary injection for a similar purpose.

Calcium also reduces or abolishes direct irritability, which again can be restored by sodium. The loss of indirect and direct irritability by calcium is not exactly parallel. Cooling seems to favor the effects also of calcium.

In these experiments neither sodium nor calcium exclusively increased or decreased the irritability. Both depressed in primary infusion and were mutually antagonistic in secondary infusions.

53 (391)

The effects of local applications of chloride and sulphate of magnesium upon the centers in the medulla compared with those of sodium chloride.

By **J. AUER** and **S. J. MELTZER.**

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

The three salts were applied in molecular solutions to the exposed medulla oblongata of rabbits. Both salts of magnesium abolished sooner or later all the functions depending upon the centers located in the medulla, the average time until a complete effect took place being fifteen minutes. Respiration stopped and

blood-pressure came down to forty or thirty millimeters of mercury and sometimes even lower. Strong stimulations of the sciatic nerve had now no effect upon blood-pressure. After curarin and strychnin were given stimulation of the sciatic caused some rise (spinal centers). Electric stimulation of the superior laryngeal nerves or mechanical stimulation of the pharynx caused no deglutition. Injection of fluid into the œsophagus caused no contraction of that organ (no secondary peristalsis). Intravenous injection or local application of calcium did not restore these functions. In a few cases spontaneous respiration returned after a few hours of continuous artificial respiration.

Sodium chloride had no depressing effect; on the contrary, there was a moderate stimulating effect upon the respiration and blood-pressure. There was a strikingly stimulating effect upon the center of deglutition; for eight or ten minutes the animal had to swallow every ten or fifteen seconds.

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Respiration by continuous intrapulmonary pressure without the aid of muscular action.

By **J. AUER** and **S. J. MELTZER**.

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

Investigations of the nature of the mechanism which keeps up the respiration in the underpressure and overpressure methods of Sauerbruch and of Brauer led to the discovery that the respiration can be kept up for hours by a continuous stream of air equal to fifteen or twenty millimeters of mercury without the aid of any muscular action. The only requirement is that the air stream must reach at least the bifurcation. If the air is introduced simply through a tracheal cannula, as in the Brauer method, and curare is given, the animal dies in a few minutes. Our object was attained in three ways. In one method a slit was made in the trachea and a glass tube, filling out about two thirds of the trachea was introduced to the tracheal bifurcation or even a short distance into the right bronchus. Air entered through this tube and returned through the slit in the trachea and through the

mouth and nose. In the second method a short tracheal cannula was tightly ligated into the upper part of the trachea and a narrow tube was introduced through a small slit in the lower part of the trachea into the right bronchus. The air entered through the tracheal cannula and had to reach the lower end of the glass tube before it could make its exit. Finally in a third method a long O'Dwyer tube bent at right angles was introduced into the larynx, the pharynx and mouth were packed with gauze, and a long soft rubber catheter was introduced through the O'Dwyer tube deep into the trachea so that its lower end reached the bifurcation. By means of a T-tube the air entered through the O'Dwyer tube into the trachea and had no other escape than through the side openings at the lower end of the catheter (the air passed through an ether bottle; the animals also received morphin). By any of these methods the animals (dogs and rabbits) continued to live for a long time after their muscular action was completely eliminated by curare. The thorax was wide open in most of the experiments and the widely distended lungs showed only the vibrations due to the heart beats. In many cases the lungs lost their pink color. Opening the ether bottle for a second or two permitted a momentary collapse of the lungs and in an instant they again looked pink.

Besides the principle which is demonstrated by this new observation and the possibility of its practical application, it offers a very convenient method for the study of the heart movements without any interference from the respiratory movements.

55 (393)

Note on the production of kidney insufficiency by reduction of the arterial circulation of the kidney.

By **ALEXIS CARREL.**

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

In order to obtain an insufficiency of the renal functions, I attempted to find a method simpler and more practical than the reduction of renal substance used by Tuffier, Bradford, Pearce and others. This new method consists of reducing the renal circula-

tion by ligature or stenosis of the branches of the renal artery. The operation is harmless and very simple. The results obtained by Dr. Janeway show that it is efficient.

56 (394)

A modification of the Riva-Rocci method of determining blood-pressure for use on the dog.

By **THEODORE C. JANEWAY.**

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

Previous studies of the blood-pressure changes in living animals, by repeated direct measurements from the femoral or carotid, while accurate from the standpoint of the blood-pressure at the moment of observation, have been of very limited value. When used as a means of following the changes occurring over long periods of time, as in the study of experimental kidney insufficiency, it is questionable whether a single pre-operative reading, with several post-operative ones, afford in themselves any basis for the conclusions drawn. The figures given by Volkmann for the blood-pressure of different animal species show readings from the dog of 104, 123, 143, 157, 166 and 172 mm., a variation so wide that, in the light of our knowledge of the fluctuations of blood-pressure in man, it suggests strongly the fallacy of any conclusions drawn from a comparison of two or three measurements at long intervals.

To obtain some more definite idea of the changes occurring from day to day in experimental animals, I have endeavored to apply to the dog the commonly employed clinical methods. After various attempts, the most satisfactory method was found to be a modified Riva-Rocci cuff applied to the lower foreleg, the pulse being palpated in the artery at the bend of the ankle or in the plantar aspect of the paw. A rubber bag 7.5 × 15 cm., with a slightly larger outer leather cuff, will fit almost any dog, the foreleg being not less than 8 cm. in length, and from 11 to 14 cm. in circumference in a large number of laboratory dogs examined. For small dogs a cuff 5 × 11 cm. is adequate. Measurements are greatly facilitated by using a pressure bottle connected with the cuff and the manometer through valves operated by foot pedals,

leaving both hands free, in place of the usual rubber bulb or Politzer bag as the source of pressure.

The greatest difficulty is the satisfactory palpation of the small pulse in the foot. In certain dogs it is impossible, and animals must be selected that possess a reasonably large and superficial artery. It is impossible to appreciate the return of the pulse after obliteration, but with practice, if the animal can be kept quiet, the obliteration of the pulse can be appreciated within perhaps 10 or 15 mm. limits of error, always on the side of under-estimation. When the foot is cold, it should be wrapped in warm cloths to dilate the vessels, before taking readings.

A number of experiments, in which I have followed the pressure changes during an operation coincidently with a direct carotid tracing, show that one can follow fairly rapid and marked fluctuations of blood-pressure in this way, with reasonable certainty. The results have no absolute, but, I am convinced, a real relative value. For the solution of such problems as the one studied by Pässler and Heineke, and which Carrel and I are engaged in, I believe that frequent approximate blood-pressure observations are of more significance than a few isolated, though accurate, measurements.

57 (395)

Note on the blood-pressure changes following reduction of the renal arterial circulation.

By **THEODORE C. JANEWAY.**

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

Of the various workers who have studied the effects of reduction of kidney substance, only Pässler and Heineke record systematic blood-pressure observations. They were able to make direct measurements in the femoral on five dogs before and after operation, and reported a rise in pressure in all, the smallest increase being 15 mm., the greatest 29 mm., and the average 21.5 mm. These figures are based on the comparison of single readings before operation with one or more after operation, and are open to the objections I have previously urged. Because of the small number of reported observations in this field, I hope to be pardoned for presenting my still very incomplete studies at this time, in order

that I may demonstrate one of the animals now living with reduced kidney substance and hypertension.

I have made blood-pressure readings, by the rough method previously described, on twenty-three dogs, over a period of fifteen months. As a guide to normal readings in the dog I have figures from twelve dogs that were in good health, several of these being finally checked by direct carotid tracings. In these twelve dogs the average pressure, calculated from a number of readings on each, lay between 91 and 119 mm., the highest individual reading being 130 mm., and the lowest 85 mm. A number of observations made before operation on the ten nephrectomized dogs showed an average blood-pressure between 90 and 117 mm.; highest reading was 135 mm., lowest 80 mm. These readings average lower than those obtained in man, but the method as applied to the dogs is more comparable to the results of Gärtner in man, since the artery used is more peripheral, and the pressure within it more subject to fluctuations due to variations in local vasomotor tone. As I have already said, however, the errors are all on the side of too low readings; therefore, with a sufficient number of pre-operative readings to give a fair average, the finding of a marked rise in blood-pressure subsequently cannot be attributed to errors inherent in the method.

The dogs studied were operated on by Dr. Carrel as already described, with the exception of one after the method used by Bradford, Pässler, and Pearce. Four died from too extreme reduction of the arterial blood supply of inanition, and one of an abscess unrelated to the operation. All showed a slight rise in pressure in the first three to seven days, with subsequent fall, except one dog that died of extensive resection in four days. The most striking of the fatal cases showed the following:

	Blood-pressure, mm. Hg.		
Dog 19.	Maximum.	Minimum.	Average.
Before operation, 15 days.....	110	100	106
After operation, 21 days.....	135	120	127
Terminal period, 14 days.....	110	70	83

Five dogs are still living, one having been operated on 105 days ago, the others a shorter time. This dog, No. 12, shows the following clear result:

	Blood-pressure, mm. Maximum.	Minimum.	Hg. Average.
Before operation, 45 days	110	80	90
After " first period, 43 days	120	100	111
" " second period, 26 days	140	110	121
" " third period, 31 days	150	110	125
" " whole period			120

He has gained from 12,770 grm. to 16,250 grm. and his urine has been free from albumin for three weeks, but is increased in quantity. A still more marked hypertension has been obtained in Dog 20, as the following table shows:

	Blood-pressure, mm. Maximum.	Minimum.	Hg. Average.
Before operation, 23 days	135	95	117
After " 35 "	175	130	150

This dog is excitable and single readings are liable to vary somewhat on this account, but the comparison of the averages, based on eleven observations before and twenty-one after operation, or of either the highest or lowest readings, all show a true hypertension as a result of the reduction of functioning kidney substance by this method. This dog has a persistent albuminuria, with casts and red blood cells, and a daily urine quantity of about 500 c.c. The three remaining dogs have not been under observation long enough, or have not had sufficient reduction of circulation, to give definite results as yet.

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The effect of experimental acute insufficiency of the right heart upon the volume of the organs.

By **H. C. THACHER, M.D.**

[*From the Laboratory of the Medical Clinic in Tübingen.*]

If a small balloon be introduced into the right auricle or ventricle, its inflation interferes with the action of the right heart and renders the heart insufficient to perform its normal work. The effect of this upon the systemic circulation should be nearly similar to that caused by acute cardiac insufficiency in general. The changes in the volumes of the brain, liver, spleen, kidney, and extremity resulting from such cardiac obstructions were registered by oncometry in rabbits, cats and dogs.

The carotid blood-pressure, which was always registered as an

index of the degree of circulatory disturbance produced, falls abruptly to a lower level when the balloon is inflated. It then remains fairly constant until a final collapse occurs just before exitus.

The liver and brain increase at once in volume as the result of an acute passive dilatation caused mechanically by the increased venous pressure. In the other organs and extremity a moderate similar passive dilatation can be demonstrated if, but only if, they have been put in a state of active contraction before the cardiac obstruction is made. Thus when previously contracted directly, by the intravenous injection of adrenalin, or reflexly, by sensory irritation, their volume-curve rises during the period of cardiac insufficiency. But unless thus previously contracted, the kidney, spleen, intestine, and extremity decrease promptly in volume when the heart is obstructed. This decrease overshadows the relatively slight effects of the increased venous pressure, so that the presence of the latter is only manifest in a short "additional fall" of the volume-curve which occurs just at the moment when the obstruction is removed. This "additional fall" is synchronous with the drop in the venous pressure, and occurs before the organs begin to return to their normal size.

The decrease in volume of these organs, on the other hand, does not correspond to the fall of arterial blood-pressure, but may continue for as much as five minutes after the latter has reached its lowest point. It is due rather to an active contraction of the arteries and capillaries tending to compensate for the blood lost from the circulation by stagnation in the veins, liver, and brain.

The foregoing work was conducted in the laboratory of the Medical Clinic in Tübingen. I desire here to express my gratitude to Professor Romberg and his first assistant, Dr. Schlayer.

Thirty fourth meeting.

The Rockefeller Institute for Medical Research. May 26, 1909. President Lee in the chair.

59 (397)

The comparative toxicity of sodium chloride and of staining solutions upon the embryo of Fundulus.

By **ELIZABETH COOKE** and **LEO LOEB.**

[*From the Laboratory of Experimental Pathology of the University of Pennsylvania; and from the Marine Biological Laboratory, Woods Hole.*]

What substances enter cells and upon what conditions the entrance of various substances into cells and the permeability of organized animal membranes generally depends, is as yet only very imperfectly understood. The following experiments may not be without interest in this connection.

In studying the toxicity of stains upon star-fish eggs, we find thionin, Bismarck brown, methylene blue and neutral red all to be very poisonous, if the solutions are exposed to light. Among these, neutral red is perhaps less poisonous than the other stains. Solutions of eosin are very much less toxic than the other substances. Thionin, Bismarck brown, methylene blue and neutral red easily penetrate into ova and stain them in a characteristic way. Eosin does not stain living cells, but only enters in combination with the dead protoplasm.

Very different is the degree of toxicity of these stains towards the eggs of *Fundulus*. Here, Bismarck brown, thionin, methylene blue and eosin are devoid or almost devoid of toxic action, whilst neutral red alone possesses any marked degree of toxicity, if the solution acts in the light. And the latter stain is likewise the only one able to enter the healthy ova of *Fundulus* and to stain certain parts of the embryo. We are therefore justified in the conclusion that in the case of stains the toxicity of these substances towards

ova is dependent upon and is an indicator of their combination with the protoplasm of certain cells of the embryo.

Now, it is not without interest to state that the toxicity of neutral red varies according to the stage of development at which the eggs are exposed to the influence of the staining solutions. Ova immersed in a solution of neutral red and exposed to the light, inside of sixteen hours after fertilization are most severely affected; ova exposed approximately twenty to thirty hours after fertilization are somewhat more resistant, and ova which are exposed to the light as late as two to four days after fertilization are affected only to a very slight degree.

Correspondingly, we find that the older the embryo becomes, the less is it liable to be stained with neutral red and in embryos five days old we usually find almost the whole embryo unstained with the exception of the newly developed liver which appears in an orange-yellow color.

We may therefore conclude that the embryos of *Fundulus* and their cells become less and less permeable for neutral red as the development advances and that its toxicity decreases correspondingly.

A curve of toxicity almost parallel to that of neutral red we find in the case of isotonic sodium chloride solutions. During the first sixteen hours isotonic solutions of sodium chloride are extremely toxic to the embryo of *Fundulus;* from twenty to thirty hours there is noticeable a certain decrease in toxicity; while embryos two to four days old develop in 5/8 N. sodium chloride solutions almost as well as in sea water. We see, therefore, that the similarity of the curves is very great and inasmuch as in the case of the neutral red the variations in toxicity seem to depend upon variations in the staining ability of this substance and therefore probably upon the permeability of certain membranes or of the protoplasm of certain cells to the stain, we may assume as the most plausible explanation that in the case of sodium chloride the variations in toxicity also depend upon the permeability of certain organized structures to the latter substance, and that therefore the conditions of permeability in the embryo of *Fundulus* depend upon the same conditions in the case of the lipoid soluble neutral red and in the case of lipoid insoluble inorganic salts, a conclusion which is at

variance with the views of Overton and Hoeber, but agrees with the observations made by Jacques Loeb, Robertson and by the botanist, Ruhland. We are well aware of the number of variable factors which are to be taken into account in the interpretation of these phenomena which may perhaps later necessitate a somewhat more complicated explanation; but we believe that comparative studies in the toxicity of stains and of various other substances will prove to be of value in the elucidation of the problems of cell permeability and of the cause of toxicity.

60 (398)

The influence of calcium chloride and of adrenalin upon the secretion of urine and upon absorption from the peritoneal cavity.

By **MOYER S. FLEISHER** and **LEO LOEB.**

[*From the Laboratory of Experimental Pathology of the University of Pennsylvania.*]

I. Intraveneous injection of calcium chloride diminishes the secretion of urine. Porges and Pribram ascribed this effect to the lowering of blood pressure which follows the intravenous injection of this substance. Our experiments, we believe, show such an interpretation to be erroneous for the following reason:

If we add adrenalin to sodium chloride solutions the blood pressure rises during the intravenous injection of this fluid and we also find a noticeable increase in diuresis under the influence of adrenalin. If we now add calcium chloride to the adrenalin-sodium chloride mixture the blood pressure remains likewise very high during the intravenous injection and the ultimate fall due to the influence of calcium chloride is delayed for a considerable time, but notwithstanding the high blood pressure produced by adrenalin which is in itself a substance favorable to diuresis, the addition of calcium chloride again causes a marked decrease in diuresis. The effect of calcium chloride in diminishing the secretion of urine can therefore not be ascribed to its action on the blood pressure, but to some other condition, most probably to its direct influence upon the epithelial cells of the kidney, an interpretation originally given by John B. MacCallum.

II. As Exner and Meltzer and Auer found, the intravaneous (Meltzer and Auer) and intraperitoneal (Exner) injection of adrenalin delays the absorption of fluoresceine and other substances from the tissues, from the peritoneal cavity and from the blood vessels. On the other hand, we found that adrenalin has a distinctly accelerating effect upon absorption of isotonic sodium chloride solutions from the peritoneal cavity, if adrenalin is injected repeatedly intraperitoneally during a period of two and a half hours. This accelerating effect is absent in nephrectomized animals; it is, however, noticeable in rabbits injected twenty hours previously with uranium nitrate and is still indicated in animals injected with uranium nitrate three days before testing the absorptive power.

Adrenalin also causes an increase in the secretion of urine and the improved absorption might therefore perhaps be ascribed to the increased elimination of fluid through the kidneys. Such an interpretation seems to be strengthened, if we consider that in nephrectomized animals this effect of adrenalin is absent. On the other hand, in individual experiments, parallelism between the absorption from the peritoneal cavity and the degree of diuresis frequently is absent. In experiments concerning the effect of coffeine upon the absorption from the peritoneal cavity we found that after injection of coffeine the absorption from the peritoneum may be very slight notwithstanding a very strong diuresis. We also notice that during the first period of the action of uranium nitrate the increased diuresis is not accompanied by a corresponding increase in absorption.

Notwithstanding these possible objections, at the present we cannot yet exclude the possibility that the improvement in absorption from the peritoneal cavity under the influence of adrenalin is due to the diuretic action of this substance. The difference in absorption from the peritoneal cavity which we notice in experiments with animals after nephrectomy on the one hand and after administration of uranium nitrate on the other hand, is of interest and may perhaps be a causative factor in the edema which develops in animals injected with uranium nitrate.

Furthermore these experiments suggest that adrenalin may improve the absorption of water, but at the same time retard the absorption of sodium chloride from the peritoneal cavity. We have begun experiments, in order to decide this question.

61 (399)

Observations on uricolysis, with particular reference to the "uric acid infarcts" of the newborn.

By **H. GIDEON WELLS** and **HARRY J. CORPER.**

[*From the Pathological Laboratory of the University of Chicago.*]

Mendel and Mitchell demonstrated that in the embryo pig the enzymes concerned with purin metabolism appear at different stages of development, the uricolytic power not appearing until after birth and being feeble during the first months of extra-uterine life. If the same late development of uricolytic power were present in the human fetus it would explain the occurrence of deposits of urates in the kidneys of newborn infants. Schittenhelm and Schmidt alone have studied uricolysis by infantile and fetal tissues, and have claimed to get active uricolysis. This result is questionable, because later work by Kunzel and Schittenhelm indicate absence of uricolysis by adult tissues. We have found no evidence whatever of uricolytic activity on the part of fetal tissues at any stage of development, nor of adult tissues. The latter observation is in harmony with the negative results obtained by Wiechowski in experiments *in vitro* and *in vivo*, and indicates that the human body has little if any power to destroy uric acid. The statements in the older literature that allantoin is found in the urine of pregnant women has been disputed by Wiechowski, and our failure to demonstrate uricolysis by human placenta as well as other fetal or adult human tissues points in the same direction.

Additional observations are the demonstration of active uricolysis by the liver of the guinea pig, absence of uricolysis by spleen, bone marrow and probably the leucocytes of the dog, and the apparent absence of inhibitory power of dog serum upon uricolysis by dog liver.

62 (400)

Studies on the life cycle of Paramecium.

By **LORANDE LOSS WOODRUFF.**

[*From the Sheffield Biological Laboratory of Yale University.*]

A year ago I reported to this society the results obtained up to that time on the life cycle of *Paramecium* when subjected to a

varied environment. I wish now to bring the results up to date (May 26, 1909).

A culture of *Paramecium aurelia* (*caudatum*) was started on May 1, 1907, with a "wild" individual isolated from a laboratory aquarium, and during the twenty-five months which have elapsed since that time it has been under daily observation. Infusions of hay and grass together with any material that may be found in the normal habitat of *Paramecium* have been employed as a culture medium. The possibility of contamination by cysts or "wild" *Paramecia* has been eliminated by boiling the infusion. Daily isolation of an individual from each of the various lines of the culture has enabled an accurate record of the division rate to be kept and has precluded the possibility of endogamous conjugation.

So far the culture has attained the 1,185th generation. The average rate of division for the entire period is over one and a half divisions per day. The average rate has not fallen during any ten-day periods as low as one division in two days, while during several ten-day periods it has averaged over two and a half divisions per day. Marked physiological depression has not been indicated by the rate of division and consequently special stimuli have not been employed to "rejuvenate" the organisms.

The results thus far obtained certainly show that the life cycle of *Paramecium* when subjected to a varied environment may be of very great duration, and, I believe, strongly suggest that the life history may be of unlimited duration.

63 (401)

Immunity to various species of trypanosomes induced in mice by the cure of experimental infections.

By **B. T. TERRY.**

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

When properly treated a temporary immunity was secured against the organisms of surra of India, surra of Mauritius, caderas, dourine and nagana. The immunity was specific in the sense that it was active against the species cured but not against any other. The immunity varied with the virus, the medicament, the time at which the immunity tests were begun, the number of the tests, the

intervals between them and the natural variations in the mice employed.

Against the parasites of surra of India, surra of Mauritius, caderas, nagana, and a toluidin blue resistant strain of nagana, an immunity was produced which was strong enough to prevent one or more subsequent inoculations of virus from infecting. The immunity to dourine was less strong.

In producing immunity to surra of India a single injection of dichlorbenzidine plus amidonaphtol disulphonic acid, 1, 8, 3, 6, or "Cl," was more efficient than one of acetylatoxyl or arsenophenylglycin.

Even when the medicaments apparently prevented infection an immunity was produced. It is interesting that two injections of a mixture of "Cl" and acetylatoxyl has completely protected a normal mouse against an inoculation with surra of India given six days after the second treatment. The mixture protected about twice as long as either of its two constituents used alone had done.

The immunity reaction distinguished with sharpness organisms supposed to have had a common origin, namely, surra of India and surra of Mauritius. Occasionally, however, these two species immunized against each other. With equal clearness the reaction distinguished between organisms known to have had a common origin, *e. g.*, the toluidin blue resistant strain and the parafuchsin resistant strain. When mice were immunized to the toluidin blue trypanosomes, an inoculation with the same virus failed completely, while the tests with the parafuchsin resistant strain and with all others, infected and killed.

By means of the immunity reaction it was apparently possible to separate in purity organisms that had been mixed *in vitro*. By inoculating a mixture of surra of India and surra of Mauritius into mice immune to surra of India an infection with surra of Mauritius was obtained. The surra of India was separated by inoculating the mixture into mice immune to surra of Mauritius. In a similar way caderas and surra of India were separated.

When mice infected with a mixture of two species of trypanosomes were cured, an immunity to both was produced.

In securing prolonged specific immunity, frequent injections of the virus at close intervals were of value. A mouse cured of

caderas by a single injection of trypanred acquired an immunity that lasted 50 days. Another mouse cured of surra of Mauritius by acetylatoxyl and "Cl" had a strong immunity. In the 46 days that followed treatment it received eleven inoculations of virulent surra of Mauritius trypanosomes but did not become infected. When next tested for its immunity (311th day), it was apparently hypersensitive to infection. It was killed in 8 days by a greatly attenuated surra of Mauritius strain. The attenuation was apparently due to long continued passage through guinea pigs. One of the two controls inoculated with this virus died on the twentieth day. The other carried the trypanosomes for 111 days, recovered spontaneously, and is still alive (184th day).

64 (402)

The leucin fraction of proteins. II.

By **DONALD D. VAN SLYKE** and **P. A. LEVENE.**

[*From the Rockefeller Institute for Medical Research, New York.*]

In a previous communication[1] we have described a method for quantitatively separating leucin and isoleucin from valin, the leucin isomers being precipitated as normal lead salts. It has since been found that from the specific rotation of the mixture of leucin and isoleucin, as obtained analytically pure from the lead salts, the percentage of each can be accurately calculated. The rotation of d-leucin in 20 per cent. hydrochloric acid is + 36.8°, of l-leucin + 15.6°. Consequently the proportions are calculated by the formulæ :

$$\text{per cent. d-isoleucin} = \frac{100\,(R - 15.6)}{21.2},$$

$$\text{per cent. d-leucin} = \frac{100\,(36.8 - R)}{21.2}$$

(R representing specific rotation in 20 per cent. hydrochloric acid). The specific rotations of the isomers are unaffected by the boiling acid used for hydrolysis, but are affected by boiling with alkalies. Consequently the method is not applicable to products of alkaline hydrolysis, which however is seldom used.

[1] These Proceedings, 1909, vi, 54.

Analysis of the leucin fractions of casein and edestin gave the following results, those obtained by Abderhalden being given for comparison. The figures represent yield in grams from 100 grams of protein.

	Casein.		Edestin.	
	V-S. and L.	Abderhalden.	V-S. and L.	Abderhalden.
Leucin,	7.84	10.5	8.1	20.9
Isoleucin,	1.51			
Valin,	6.69	1.0	5.6	0.45
Total "leucin fraction,"	16.04	11.5	13.7	21.35

Our figures are based on analytically pure products. It is evident that Abderhalden missed from 80 to 90 per cent. of the valin which was undoubtedly calculated in with the leucin, as the two are not separable by the methods previously available. Still other products in the necessarily crude mixture may account for the high yield from edestin.

65 (403)

"Clavin," Vahlen's active principle of ergot.

By **DONALD D. VAN SLYKE.**

[*From the Rockefeller Institute for Medical Research, New York.*]

In a recent paper Vahlen[1] describes the isolation of the two constituents of clavin, a crystalline substance isolated by him from ergot. By means of their copper salts two substances, leucin and "clavin base" of the formula $C_5H_{11}O_2N$, were separated from the clavin. From analysis of clavin it appeared to consist of leucin and clavin base in molecular proportions, and was regarded as a salt-like combination, similar to that in which some alkaloid bases are found, the leucin acting as acid, the clavin base as base. Vahlen regards clavin as the active principle of ergot in stimulating contraction of the uterus, and the clavin base as the active constituent of clavin.

From the description of clavin, it appeared similar in both physical and chemical properties to the mixture of leucin and valin obtained from proteins. A sample of Vahlen's clavin was obtained from Merck and submitted to the process devised for determination of leucin, isoleucin, and valin in the presence of one another.[2] The

[1] *Arch. f. exper. Path. u. Pharm.*, 1909, lx, 42.

[2] Levene and Van Slyke: These Proceedings, 1909, vi, 54.

clavin, which when purified free from ash had the properties described by Vahlen, consisted entirely of these three amino acids, the latter being isolated analytically pure: 2.02 grams of clavin gave 0.79 gram of leucin, 0.45 gram isoleucin, 0.75 gram valin. We have not yet determined whether any of these amino acids has the pharmacological effect assigned to clavin.

66 (404)

Some effects of sodium benzoate.

By **DANIEL R. LUCAS.** (By invitation.)

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons.*]

This research was suggested to me by different experiences with sodium benzoate when taken by mouth in the following ways: A. Pure (1) as crystalline salt, or (2) in aqueous solution. B. In neutral or alkaline solutions, or in mixtures rich in fat, carbohydrate or protein, *e. g.*, milk. C. With vegetable or fruit acids (1) hot, as in tomato soup, or (2) cold, as in canned plums, oranges, lemons, etc. D. In beverages containing high percentages of organic acids, *e. g.*, cider, lemonade, grape juice, wine, etc. E. In mixtures containing inorganic acids, *e. g.*, artificial gastric juice.

Brunton has studied the effects of benzoic acid on enzymes and bacteria. The strong inhibiting effect of this substance on their activity is in striking contrast to the slight effect of sodium benzoate. Doepner has shown that fairly large quantities of sodium benzoate (2 per cent.) did not prevent the development of *Proteus vulgaris* and, in strengths equal to 0.5 per cent., only slightly retarded the development of *B. enteritidis*, *B. fluorescens* and *B. coli*. Fleck found that benzoic acid in concentrations equal to 0.6–0.7 per cent. caused marked inhibition of yeast fermentation and that the inhibiting action was markedly decreased by the amount of protein present. Lehman observed that meat extract putrefies in the presence of 1 to 2 per cent. of sodium benzoate, but less benzoic acid acts more strongly antiputrefactive when the reaction of the medium is markedly acid. The action of sodium benzoate under markedly acid conditions is the same as the action of benzoic acid. Under such conditions the action of the benzoate diminishes with decrease of

acidity. Chassevant and Garnier found that 1.4 gram of benzoic acid per kilo was fatal to guinea pigs in five to seven hours; larger doses (2 grams per kilo) did not necessarily kill sooner.

The results of my own work may be briefly summarized as follows:

Effects on microörganisms.—Sodium benzoate, in concentrations of about 1 per cent., preserves fruits and vegetables which are *strongly* acid. Crystals of free benzoic acid often appear in such mixtures. Sodium benzoate (1 per cent.) added to *weakly* acid fruits and vegetables does not preserve them well. Sodium benzoate (1 per cent.) added to fruits and vegetables, the acidity of which has been *neutralized*, does not preserve them. Pure apple juice, containing 0.1 per cent. of sodium benzoate, developed mould after ten days; commercial benzoated cider under the same conditions, without the further addition of benzoate, did not develop mould or otherwise undergo degeneration.

Effects on taste. — Acid fruit juices containing 1 per cent. of sodium benzoate, have a biting taste, an effect due to the liberated benzoic acid. Milk or alkaline vegetables treated with sodium benzoate (1 per cent.) do not taste of benzoic acid at any time during the first twenty-four hours after the treatment. After twenty-four hours, however, acid decomposition begins in milk in spite of the presence of 1 per cent. of sodium benzoate, when the mixture tastes distinctly of benzoic acid. Sips of 1 c.c. of orange juice, to which 1 per cent. of sodium benzoate has been added, cause burning in the posterior part of the mouth, the throat, the esophagus and stomach, with gastric discomfort, belching, uneasiness of the bowels and excessive passage of gas by rectum.

Experiments on men with cider. Pure cider. — Pure apple juice tastes sweet, bland; produces no stinging sensation in the throat; and is ordinarily enjoyed and well borne in volumes equal to 1,000 to 2,500 c.c. (ingested during a period of two or three hours). It is diuretic in action and, in amounts varying from 1,000 to 2,000 c.c., causes laxation of the bowels. This effect frequently depends on the rapidity with which it is ingested; it does not ordinarily cause laxation even when taken in large amounts, if ingested little by little. The average amount of pure apple juice consumed during an evening by adult males who

had free access to it was about 1,200 c.c. (twenty subjects). When from 1,000 to 2,500 c.c. of pure apple juice are consumed neither headache, nausea, albuminuria nor sub-normal temperature is produced. The specific gravity of the urine is greatly decreased when a liter of pure unfermented cider is consumed but the volume is markedly increased within forty-five minutes after its ingestion. The forty gallons of pure apple juice consumed by the human subjects of my investigation contained considerable apple pulp and 2.716 grams of free acid (calculated as acetic acid) per 1,000 c.c.

Benzoated cider. — Twenty four subjects were observed in the first experiment. Twelve received *pure* apple juice; twelve received samples of the same apple juice containing 0.1 per cent. of sodium benzoate. As none of the subjects knew that they were to receive at that time anything but pure apple juice, unfavorable psychological influences were eliminated from the experiments. Each subject received three question blanks to be filled out by himself daily as long as any symptoms lasted, which, I am assured, was done faithfully in every instance.

In comparison with those who received pure cider, the men who drank the benzoated apple juice exhibited the following special symptoms: Burning taste, fulness in the head, headache, nervousness, nausea, vomiting, itching of the skin, unusual perspiration, irregularity of bowels (*constipation* usually) *decreased* flow of urine, *increased* specific gravity of the urine, and albuminuria. Excessive amounts of hippuric acid were eliminated, especially during the first few hours, after ingestion of the benzoated apple juice.

Apple juice to which a small amount of sodium benzoate is added becomes sweeter to the taste, but astringent, stinging, and irritating to mucous membranes. The presence of 0.5 per cent. of sodium benzoate renders cider quite unpalatable, but the presence of 0.1 per cent. may be overlooked by subjects not acquainted with the taste of pure apple juice.

If the apple pulp is previously filtered from the juice the effects of added benzoate become much more evident. A liter of such filtered cider, containing 0.2 to 0.3 per cent. of sodium benzoate, caused albuminuria within three hours almost without exception in the largest and soundest picked subjects. However, I myself

was able to ingest 1,000 c.c. of apple juice containing 0.5 per cent. of sodium benzoate, without any albuminuria arising. The amount of hippuric acid in the urine was very large for the first few hours. The secretion of urine was very much reduced for twelve hours, while I suffered from some of the other symptoms above mentioned, *although as a subject in a former investigation I ingested as much as 6 grams per day, for three successive days, in milk on a full stomach, without the slightest discomfort.*

Small doses of sodium benzoate given with *acid* substances to patients with albuminuria aggravated this condition and caused alarming symptoms, classical of nephritis — for six days thereafter in one subject.

Experiments on dogs. First experiment. — One dog weighing 3.5 kilos fasted for twenty four hours and was then given 1 gm. of sodium benzoate, decomposed with the theoretical amount of hydrochloric acid to form sodium chloride and free benzoic acid, with no excess of hydrochloric acid. In thirty minutes the animal showed evidences of muscular weakness and nausea, lay quietly and breathed in a laborious manner. This continued for six hours. On the next day, twenty four hours after the previous dose, the animal was given 4 gm. of sodium benzoate with a sufficient amount of hydrochloric acid to decompose it into benzoic acid, plus 120 c.c. of 0.2 per cent. citric acid. The animal became very weak in one hour, respirations were reduced to nine per minute, and were very labored. Tonic and clonic convulsions began one hour and fifteen minutes after the dose was given. The animal, after several hard convulsions, died two hours and twenty minutes after administration.

The autopsy showed congestion of various organs. There was very pronounced congestion of the kidneys, stomach and intestines, with ulceration in places. The liver and lungs also showed evidences of infarcts.

Second experiment. — Two dogs had been fed on dog biscuits and water for several weeks, and then fasted for thirty six hours.

Animal No. 1. — The first animal was a male, weighing 3.5 kilos. He was given a mixture of 3.5 gm. of sodium benzoate, 50 c.c. of water, 0.65 c.c. of concentrated hydrochloric acid (sp. gr. 1.19) and 100 c.c. of citric acid (0.2 per cent.). The animal

became quite uneasy after receiving the dose. At the end of an hour he showed great muscular weakness and tremor.

Animal No. 2. — The weight of the second animal was 4.25 kilos. It was given a mixture of 100 c.c. of 0.2 per cent. citric acid, 50 c.c. of water and 0.85 c.c. of concentrated hydrochloric acid (sp. gr. 1.19). This animal was entirely unaffected.

The same experiments were repeated on the same animals the next day; the results were practically identical. The animal (1) that received the free acid-benzoic acid mixture, however, was more prostrated than on the previous day and showed general stiffness of the muscles. At the end of six hours it was chloroformed and autopsied, when it was found that the stomach contained "coffee ground" material. There were ecchymotic areas and some places appeared to be ulcerated slightly. The intestines showed marked congestion here and there and appeared to be slightly ulcerated in places throughout. The grumous material in the stomach and intestines gave a strong guaiac test and was undoubtedly modified blood. The liver and lungs showed considerable congestion with some evidences of infarcts. The kidneys were cyanotic, the cortex very much congested, while the medulla was pale and anemic.

Further investigation is contemplated, especially on the influence of nephrectomy on the toxicity of benzoic acid.

I am indebted to many members of the Purdue University Alumni Association of New York City for volunteering as subjects in this investigation and thus making it possible for me to carry out experiments on a large number of individuals. The Secretary, Mr. Leslie Hustable, Mr. Ray C. Ewry, Mr. R. W. Parks, Mr. F. M. Waltz and Mr. H. Worsham of that organization have given me special assistance in various ways. I am also indebted to Drs. A. E. Olpp and Matthew Steel, and Messrs. Herzfeld and Bisch for coöperation, and to Drs. Foster, Mosenthal and Rosenbloom for criticism and suggestions. Professor Gies has given me all the facilities of his laboratory for the conduct of this research, as well as valuable criticism and suggestions.

67 (405)

An improvement of the Folin method for the determination of urinary ammonia nitrogen.

By **MATTHEW STEEL.** (By invitation.)

[*From the Laboratory of Biological Chemistry of Columbia University, at the College of Physicians and Surgeons.*]

In the fall of 1907, during the progress of a metabolism research on "the influence of magnesium sulphate on metabolism," [1] anomalous results were obtained in our quantitative determinations of urinary ammonia, whenever the magnesium salt was injected either subcutaneously or intravenously into the animal.[2] These anomalous results were found to be due to the facts that the magnesium was eliminated into the urines in question in relatively large quantities, as ammonio-magnesium phosphate and that the resultant deposits of crystalline triple phosphate were not thoroughly decomposed by sodium carbonate, as used in the Folin method, whereby ammonia, in variable amounts, remained in its solid form as triple phosphate in the urines under investigation. We, therefore, sought another method that would liberate all the ammonia from ammonio-magnesium phosphate without producing ammonia from such compounds as urea in the urine. None, however, was found that fulfilled both conditions. Consequently, we were obliged either to devise a new method, or else modify the Folin process, so as to make it liberate the ammonia from triple phosphate. We chose the latter course.

Our attempt was to find some alkali which would liberate all the ammonia from ammonio-magnesium phosphate and which at the same time would not convert into ammonia any of the amino or imino radicals in the various organic compounds in the urine.

Varying quantities of milk of lime, baryta water, and sodium hydroxide were added separately to weighed amounts of triple phosphate. These mixtures were aërated as usual. The results obtained made it evident that neither milk of lime nor baryta water,

[1] Steel: These Proceedings, 1908, v, 132; *Journal of Biological Chemistry*, 1908, v, 85.

[2] Steel and Gies: These Proceedings, 1908, v, 134; *Journal of Biological Chemistry*, 1908, v, 71.

even in large amounts, was capable of liberating all the ammonia from triple phosphate, whereas sodium hydroxide, in comparatively small amounts, discharged the ammonia completely. The first condition was, therefore, solved. It now remained for us to ascertain whether sodium hydroxide would produce ammonia from such amino compounds as urea.

After many preliminary trials, in some strictly comparative tests on normal urines, I found that from 0.5 gram to 1 gram of sodium hydroxide, plus about 16 grams of sodium chloride, gave results that were in perfect accord with those of the Folin method. Over fifty comparisons were made.

In oder to ascertain positively whether the modified method would produce ammonia from non-ammoniacal radicals, weighed samples of urea, uric acid, glycocoll, taurin, leucin, tyrosin and hippuric acid were added separately and also collectively to 25 c.c. portions of urine and the results compared with the figures for the ammonia obtained from equal volumes of the original urine. In no case was any increase obtained. The tests were repeated, but 20 c.c. of a standard solution of ammonium chloride were substituted for the 25 c.c. of the urine. In these cases, also, no increase of ammonia output was obtained.

As a final test of the efficiency of the modified method the ammonia content was determined in a normal urine by both the Folin method and the modified method. Then to equal volumes of the original urine 0.5 gram samples of triple phosphate were added, and the ammonia contents again determined by both methods. The ammonia was also determined in separate portions of the triple phosphate.

The results of these directly comparative tests showed that, *with the modified method*, the ammonia obtained from the urines to which the triple phosphate had been added exactly equaled the total amount of ammonia obtained from the corresponding urine and the triple phosphate separately whereas, *with the Folin method*, only from 70 to 80 per cent. of the total amount of this ammonia was recovered.

68 (406)

The depressor substance of dog's urine; its disappearance in experimental acute nephritis.

By **RICHARD M. PEARCE.**

[*From the Carnegie Laboratory of the New York University and Bellevue Hospital Medical College.*]

The urine of a normal dog when injected intravenously into another dog in doses of three cubic centimeters causes an immediate fall in blood pressure varying from 25 to 96 mm. of Hg. This effect, constant for normal urine, is not always obtained when the urine from a chromate or uranium nephritis of the third to fifth day is used. It is still obtained, however, in arsenic and cantharidin nephritis of the same periods. This difference suggests that in the tubular lesions of chromate and uranium nephritis, which are characterized by extensive epithelial destruction, some substance normally eliminated is retained while in the glomerular nephritis caused by arsenic and cantharidin poisoning this retention does not occur. The elimination of the depressor substance would appear therefore to be a function of the tubular epithelium.

In animals with experimental nephritis of the tubular type the disappearance of the depressor substance from the urine is frequently associated with a lowering of the blood pressure which would appear to indicate that the retained depressor substance has a definite effect on the general blood pressure. This observation is not based however on blood pressure determinations on the same animal before and after the development of nephritis but by contrasting the pressure in animals with tubular nephritis with that of normal animals and those with glomerular nephritis.

The nature of the depressor substance has not been determined.

69 (407)

Observations on the metabolism of a subject of diabetes.

By **PHILIP A. SHAFFER.**

[*From the Laboratory of Pathological Chemistry, Department of Experimental Pathology, Cornell Medical College.*]

The subject of the observations was a patient in the service of Dr. Warren Coleman in Bellevue Hospital. Different known

diets were used and the urine was analyzed over a period of more than three months. A portion of the results of the last part of the period is given in the accompanying table. The unusual features of these results will be briefly discussed.

Between twenty and eighty grams of *total* β-oxybutyric acid (the acetone and diacetic acid being calculated as β-oxybutyric acid) were excreted each day for at least eighty days without any signs of impending coma. For more than two months with this severe acidosis the subject showed practically complete carbohydrate intolerance. As shown in the table the diet was then changed to 255 grams of oatmeal. On this diet the acidosis and the glycosuria decreased very much, the former ultimately disappearing. This comparatively sudden transition from a condition of severe to one of mild diabetes is very striking and appears to confirm the good results obtained by von Noorden and others with his so-called "oat cure."

The dependence of β-oxybutyric acid production upon the amount of fat in the food was very clearly shown throughout the observations. The amount of total β-oxybutyric acid excreted varied in general with the amount of fat eaten, which is not usually the case. Note the effect of the addition of butter to the oatmeal diet. The amount of food-fat appears to have determined, in this instance, the amount of fat burned.

An increase in the amount of fat in the food which caused an increased acidosis appeared to result also in a damage to the carbohydrate tolerance ; with the increase of acidosis there was a very marked decrease in the amount of sugar burned. This phenomenon is just the reverse of that so often observed, *i. e.*, the decrease of acidosis with an increased burning of sugar, and again emphasizes the close inter-dependence between the metabolism of carbohydrate and fat.

The observations were terminated by the death of the patient. The immediate cause of death was not definitely established but was probably due to the phthisis which complicated the diabetes. There was no sign of coma and in view of the absence of acidosis and of the great improvement in carbohydrate tolerance we can scarcely believe that the diabetes was immediately responsible. The blood obtained at autopsy contained only traces of β-oxybutyric acid, the whole blood containing about 0.3 gram. There was no

suppression of urine, over 500 c.c. being passed in the last five hours.

The urine of the last two days is of interest; there was no acetone and no increase in glycosuria, but a very great increase in ammonia and in total nitrogen indicating a marked ante-mortem increase in protein katabolism.

Sugar was determined by titration with Fehling's or Pavy's solutions; and β-oxybutyric acid and its derivatives by the writer's method.

	Food.		Urine.			
	NH.	Fat.	N.	NH_3—N.	Sugar.	Total β-oxybutyric acid.
Average for 5 days.....	256	164	19.0	4.6	328	19.0
Average for 2 days.....	180	187	19.0		293	24.7
255 gm. oatmeal.....	165	18				
	"	"	7.0	1.8	92	1.7
	"	"	7.1	1.5	94	1.6
	"	"	5.8	1.1	72	0.9
Same, + 50 gm..	"	60	5.1	1.2	85	5.1
	"	"	6.6	2.3	119	11.3
	"	"	6.0	2.65	119	10.3
	"	"	5.5	2.0	108	4.9
Same, washed butter....	"	"	4.6	1.8	64	1.4
	"	"	5.6	0.9	48	0.7
	"	53	6.2	0.8	34	0.6
Same, regular butter.....	"	60	5.6		5	0.0
	"	"	6.4		4	0.4
	"	"	5.9	0.36	17	0.0
	"	"	6.7	0.83	8	0.0
Toast, butter, cane sugar,	170	59	13.4	1.75	16	0.0
eggs.....	less	less	32.2	3.32	8	0.0

Died at 1 P. M. next day.

70 (408)

On the decomposition of caffeine in the liver.[1]

By **W. O. EMERY** and **WILLIAM SALANT.**

[*From the Laboratory for Synthetic Products and Pharmacological Laboratory of the Bureau of Chemistry, U. S. Dept. of Agriculture, Washington, D. C.*]

The earlier workers on the metabolism of caffeine and theobromine maintained that these substances may undergo partial or complete transformation in the body with the loss of one or more of the methyl groups. Investigations carried out recently seemed to indicate that this was due to specific enzymes. Schittenhelm, Brugsch and Pincussohn[2] claimed to have found an enzyme in the lungs of the horse capable of splitting off the methyl groups of caffeine. Kotake[3] came to similar conclusions as a result of studies on the decomposition of caffeine in beef livers. He added varying amounts of caffeine to aqueous extracts of the liver which he allowed to digest under antiseptic precautions at body temperature in the thermostat for four days. Liver extracts without caffeine, similarly treated, were used as controls. At the end of each period the purin bodies were precipitated and total nitrogen determined. He found in every case much larger amounts of total nitrogen in the extract containing caffeine than in the control, from which he concluded that the increase of purin substances was due to the reduction of caffeine to non-methyllated purins.

The work of Fuijitani[4] has shown that caffeine stimulates peptic digestion *in vitro*. The possibility of a stimulating action of caffeine on intra-cellular enzymes is therefore not to be excluded, and might explain the results of Kotake. Moreover, in Kotake's experiments no separation of the alkaloid was attempted.

We therefore carried out a series of experiments in which the caffeine as well as the purin nitrogen was determined. Finely minced fresh beef livers were allowed to stand twenty four hours in the presence of 5 c.c. toluol and were filtered through paper.

[1] Published by permission of the secretary.

[2] *Cent. f. d. ges. Physiol. u. Path. des Stoffewechs*, 1908, ix, 290.

[3] *Arch. internat. d. pharm. et de ther.*, 1905, xiv, 21.

[4] *Zeit. f. physiol. Chem.*, 1908, lix, 378.

Half a gram of caffeine was added to a portion of the extract thus obtained, and kept in the thermostat at body temperature for from two to ten days. An equal quantity of the extract without adding caffeine, similarly treated, was used as a control. As indicated in the accompanying table, practically the entire amounts of caffeine were regained. The total quantities of nitrogen found in the precipitated purins were approximately equal save in two experiments in which the amounts of total nitrogen were from 13 to 19 per cent. greater when caffeine was added than in the controls.

Digestion of liver extracts with and without caffeine in the presence of hydrogen peroxide likewise failed to indicate the presence of a specific enzyme capable of splitting off the methyl group in caffeine.

Experiments.

Series No.	Experiment No.	Liver extract in c.c.	Duration of digestion in days.	Caffeine added.	Nitrogen.[1]	Caffeine regained.
I.	1	250	Two.	0.5	0.0699	0.4935
		250	Two.	—	0.0704	—
	2	250	Four.	0.5	0.0660	0.4921
		250	Four.	—	0.0581	—
	3	250	Eight.	0.5	0.0531	0.4915
		250	Eight.	—	0.0522	—
II.	1	300	Five.	0.5	0.0511	0.4918
		300	Five.	—	0.0499	—
	2	300	Ten.	0.5	0.0604	0.4799
		300	Ten.	—	0.0496	—
III.	1	250	Four and one half.	0.5	0.0345	0.4897
		250	Four and one half.	—	0.0370	—
IV.	1	500+ 5 c.cm. H_2O_2	Four and one half.	0.5	0.0416	0.4951
		500+ 5 c.cm. H_2O_2	Four and one half.	—	0.0441	—
V.	1	300	Four.	0.5	0.0478	0.4931
		300	Four.	0.5	0.0432	0.4952
		300	Four.	—	0.0444	—

[1] Total nitrogen was determined by E. C. Trescott.

71 (409)

The comparative toxicity of ethyl and amyl alcohol and their effect on blood pressure.[1]

By **WILLIAM SALANT.**

[*From the Pharmacological Laboratory, Bureau of Chemistry, U. S. Dept. of Agriculture.*]

The experiments were carried out on frogs, rabbits, cats and dogs. The alcohols were administered in various concentrations and were given by mouth, injected subcutaneously, or into the peritoneal cavity. The toxicity of amyl alcohol was in all cases much greater than that of ethyl alcohol. The difference in the toxicity of ethyl and amyl alcohol was even more marked in subacute intoxication. The experiments on frogs showed that the minimum fatal toxic dose of amyl alcohol is from one eighth to one seventh that of ethyl alcohol, while the toxic dose of amyl alcohol for the rabbit is only about one fourth to one half that of ethyl alcohol.

The effect of ethyl and amyl alcohol on blood pressure.— The experiments were carried out with 2 per cent. solutions on healthy dogs 8 to 10 kilos in weight, and on cats. Morphine-ether narcosis was employed for the dogs, and ether alone for cats. Injections were made from a burette into the femoral vein. The fall of blood pressure after amyl alcohol was introduced, was considerably greater than that after the introduction of the same quantity of ethyl alcohol. In some experiments, the injection of 15 c.c. of 2 per cent. amyl alcohol in thirty two seconds caused a fall of blood pressure of 80 millimeters of mercury, while the same amount of ethyl alcohol injected in four seconds was followed by a fall of blood pressure, amounting only to 20 millimeters of mercury. In other experiments in which from 25 to 50 c.c. of 2 per cent. ethyl alcohol caused little noticeable change or only a slight fall of blood pressure, after the injection of the same quantities of amyl alcohol, the maximum fall of blood pressure amounted to 40 and 95 millimeters of mercury. The recovery was also much slower in all cases after amyl alcohol and was much more gradual than the fall of blood pressure. Experiments with from 3 to 11 c.c. of 2 per cent.

[1] Published by permission of the secretary.

amyl alcohol carried out on cats has likewise shown a depressing action on blood pressure, while 25 c.c. of ethyl alcohol failed to show an appreciable change. Very small quantities of amyl alcohol (3 c.c. of a 2 per cent. solution) failed to reduce blood pressure in dogs; with larger quantities of it (10 c.c.) the fall of blood pressure was 8 millimeters of mercury, when injected in one hundred seconds. The same amount, however, when injected in seven seconds lowered the blood pressure 50 millimeters of mercury. After section of both vagi in dogs, the action of amyl alcohol was not constant; in two experiments the fall was greater, in one it was less, than with the vagi intact. The action of ethyl alcohol under these conditions likewise varied. In one experiment, amyl alcohol, 15 c.c. of a 2 per cent. solution, was injected after the introduction of atropine sulphate, both vagi being cut; the fall of blood pressure was not as great as before the injection of atropine with vagi cut, but the recovery of blood pressure to the same height as it was before the introduction of atropine occurred in from two to five minutes as against 35 seconds during the control period.

In this connection, it might be mentioned that some observations on the effect of caffeine on the depressing action of alcohol, amyl and ethyl, have been made. In both instances, there was a marked retardation of recovery of blood pressure. After the injection of 25 to 50 c.c. of 2 per cent. solutions of caffeine, the recovery was delayed, fifteen or twenty minutes.

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Pentosuria.

By **L. B. STOOKEY.**

[*From the Physiological Laboratory of the University of Southern California.*]

During the past two years one hundred urines which reduced Fehling's solution slightly were examined for identification of the reducing substance. In fifteen cases pentose was found to be present. Identification was made by (1) phenyl pentosazone crystals, (2) phloroglucin reaction, (3) absorption spectrum. The nature of the pentose was not determined. In all cases several specimens were examined under dietetic precautions in order to exclude alimentary pentosuria. In these fifteen cases no carbo-

hydrate other than pentose could be detected. The pentose content ranged between 0.1 and 0.5 per cent. In all of these fifteen urines containing pentose acetone was found in appreciable quantity. Acetone was detected by treating the distillate with sodium nitroprusside and ammonia.

Five of these fifteen cases showed a positive tuberculin reaction (subcutaneous injection of the bacillen emulsion), three gave a history of chronic alcoholism but claimed to have been abstaining for a considerable time, three were suffering from some obscure intestinal disturbances, and in regard to the remaining four cases little can be said at the present writing.

The points of interest to me are (1) apparent frequency of pentosuria, (2) positive tuberculin reaction in one third of my cases of pentosuria, (3) presence of acetone in every pentose-containing urine examined.

RECAPITULATION OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE COMMUNICATIONS.

VOLUME VI.

Carrel, Alexis

355. Presentation of a dog ten months after double nephrectomy and replantation of one kidney.

393. Note on the production of kidney insufficiency by reduction of the arterial circulation of the kidney.

Clowes, G. H. A.

354. A critical study of the conditions under which zymase and its associated co-enzyme bring about alcoholic fermentation.

Cooke, Elizabeth [with **Leo Loeb.**]

397. The comparative toxicity of sodium chloride and of staining solutions upon the embryo of *Fundulus*.

Corper, Harry J. [with **H. Gideon Wells.**]

399. Observations on uricolysis, with particular reference to the "uric acid infarcts" of the newborn.

Crile, George W.

340. Further observations on the clinical aspects of hemolysis.

Elsberg, Charles A.

382. A skin reaction in carcinoma from the subcutaneous injection of human red blood cells.

Emery, W. O. [with **William Salant.**]

408. On the decomposition of caffeine in the liver.

Famulener, Eugene [with **William H. Park.**]

386. Toxin-antitoxin mixtures as immunizing agents.

Fleisher, Moyer S. [with **Leo Loeb.**]

398. The influence of calcium chloride and of adrenalin upon the secretion of urine and upon absorption from the peritoneal cavity.

Flexner, Simon [with **Richard V. Lamar.**]

388. The action of soaps on the pneumococcus.

Foster, Nellis B.

365. Studies of the influence of various dietary conditions on physiological resistance. 1. The influence of different proportions of protein in the food on resistance to the toxicity of ricin and on recuperation from hemorrhage.

373. [With **James C. Greenway.**] Synthesis of uric acid.

Gay, Frederick P.

371. A carcinoma of the rat [Flexner-Jobling] considered from the standpoint of immunity.

390. [With **S. J. Meltzer.**] The influence of sodium and calcium upon direct and indirect muscle irritability and their mutual antagonistic actions.

Kerr, Josephine E. [with **W. J. MacNeal** and **Lenore L. Latzer.**]

379. The daily excretion of bacteria in the feces of healthy men.

Kristeller, L. [with **D. Manson** and **P. A. Levene.**]

375. On nitrogenous metabolism in chronic nephritis.

Lamar, Richard V. [with **Simon Flexner.**]

388. The action of soaps on the pneumococcus.

Latzer, Lenore L. [with **W. J. MacNeal** and **Josephine E. Kerr.**]

379. The daily excretion of bacteria in the feces of healthy men.

Levene, P. A.

346. [With **D. D. Van Slyke.**] On plastein.

361. [With **D. D. Van Slyke.**] The quantitative separation of leucin from valin.

362. [With **W. A. Jacobs.**] Further studies on the constitution of inosinic acid.

375. [With **L. Kristeller.**] On nitrogenous metabolism in chronic nephritis.

380. [With **Walter A. Jacobs.**] Further studies on the constitution of inosinic acids.

402. [With **D. D. Van Slyke.**] The leucin fraction of proteins. II.

Levin Isaac

343. A clamp for direct transfusion of blood. A demonstration.

Lewis, Paul A.

370. The influence of temperature on hemolysis in hypotonic solutions.

Lillie, Ralph S.

363. The significance of changes in the permeability of the plasma-membrane of the living cell in the processes of stimulation and contraction.

Loeb, Jacques

377. On the fertilizing and cytolytic effect of soap.

Noguchi, Hideyo

360. The butyric acid reaction for syphilis in man and in the monkey.

374. Some critical considerations on the serum diagnosis of syphilis.

Novy, F. G.

351. Successful canine infection with cultures of *Leishmania infantum* (Ch. Nicolle).

Ott, Isaac [with **John C. Scott.**]

347. The action of bile and some of its constituents upon intestinal peristalsis and the circulation.

Park, William H. [with **Eugene Famulener.**]

386. Toxin-antitoxin mixtures as immunizing agents.

Pearce, Richard M.

384. An experimental study of the influence of kidney extracts and of the serum of animals with renal lesions upon the blood pressure.

406. The depressor substance of dog's urine; its disappearance in experimental acute nephritis.

Peskind, S. (By invitation.)

394. Hemolysis in the sera of carcinoma and syphilis.

Ringer, A. I.

341. [With **Graham Lusk.**] The behavior of alanin in metabolism.

367. (By invitation.) Studies on the effects of carbon monoxide poisoning.

Robertson, T. Brailsford [with **Theodore C. Burnett.**]

378. On the depression of the freezing point of water due to dissolved caseinates.

Rosenau, M. J. [with **John F. Anderson.**]

381. The effect of heat on the anaphylactic properties of proteins.

Salant, William

408. [With **W. O. Emery.**] On the decomposition of caffeine in the liver.

409. The comparative toxicity of ethyl and amyl alcohol and their effect on blood pressure.

Salomon, Hugh [with **George B. Wallace.**]

368. Intestinal excretion during diarrhea.

Schultz, W. H. (By invitation.)

350. The effect of instilling adrenalin chloride into the mammalian eye.

Scott, John C. [with **Isaac Ott.**]

347. The action of bile and some of its constituents upon intestinal peristalsis and the circulation.

Shaffer, Philip A.

407. Observations on the metabolism of a subject of diabetes.

Shaklee, A. O.

357. [With **S. J. Meltzer.**] The mechanical destruction of pepsin.

372. Influence of temperature upon pepsin.

398. [With **S. J. Meltzer.**] The influence of shaking upon trypsin and rennin and a comparison of this influence with that upon pepsin.

Steel, Matthew.

405. An improvement of the Folin method for the determination of urinary ammonia nitrogen.

Stookey, L. B.

410. Pentosuria.

Terry, B. T.

401. Immunity to various species of trypanosomes induced in mice by the cure of experimental infections.

Thacher, H. C. (By invitation.)

396. The effect of experimental acute insufficiency of the right heart upon the volume of the organs.

Van Slyke, D. D.

346. [With **P. A. Levene.**] On plastein.

361. [With **P. A. Levene.**] The quantitative separation. of leucin from valin.

402. [With **P. A. Levene.**] The leucin fraction of proteins. II.

403. "Clavin," Vahlen's active principle of ergot.

Wallace, George B. [with **Hugh Salomon.**]

368. Intestinal excretion during diarrhea.

Weil, Richard

359. On the specific acquired relations of red blood cells.

Weinberger, William. (By invitation.)

342. An important source of error in Heller's test for urinary protein.

Wells, H. Gideon

339. Studies on the chemistry of anaphylaxis.

399. [With **Harry J. Corper.**] Observations on uricolysis, with particular reference to the "uric acid infarcts" of the newborn.

Woodruff, Lorande Loss

400. Further studies on the life cycle of *Paramecium.*

EXECUTIVE PROCEEDINGS.

Thirtieth meeting.

College of Physicians and Surgeons, Columbia University, October 21, 1908. President Lee in the chair.

Members present: Alsberg, Atkinson, Auer, Banzhaf, Burton-Opitz, Crile, Dakin, Ewing, Famulener, Flexner, Gies, Harris, Jobling, Joseph, Kast, Lee, Levene, Levin, Lusk, Mandel (A. R.), Meltzer, Meyer, Morgan, Noguchi, Opie, Park, Pearce, Shaffer, Terry, Van Slyke, Weil, Wells, Wolf.

Members elected: C. C. Guthrie, E. P. Lyon, Mazÿck P. Ravenel.

Thirty first meeting.

The Rockefeller Institute for Medical Research, December 16, 1908. President Lee in the chair.

Members present: Atkinson, Auer, Beebe, Burton-Opitz, Calkins, Carrel, Clowes, Elsberg, Emerson, Ewing, Famulener, Foster, Gies, Halsted, Hatcher, Jacobs, Janeway, Joseph, Kast, Lee, Levene, Levin, Lewis, Lusk, Meltzer, Meyer, Morgan, Noguchi, Opie, Pearce, Sherman, Terrey, Torrey, Van Slyke, Wadsworth, Weil, Wood.

Members elected: Albert C. Crawford, W. H. Schultz, Thomas A. Storey.

Thirty second meeting.

New York University and Bellevue Hospital Medical College, February 17, 1909. President Lee in the chair.

Members present: Alsberg, Atkinson, Auer, Banzhaf, Beebe, Berg, Burton-Opitz, Ewing, Famulener, Foster, Gay, Gies, Henderson, Jacobs, Joseph, Kast, Lee, Levene, Levin, Lewis, Lusk, Mandel, Meltzer, Meyer, Murlin, Noguchi, Opie, Pearce, Storey, Terry, Wallace, Weil.

Members elected: John F. Anderson, T. G. Brodie, L. J. Cole, Martin H. Fischer, Richard V. Lamar, Max Morse, Hans Zinsser.

Officers elected: President, Frederic S. Lee; Vice-President, William J. Gies; Secretary, Eugene L. Opie; Treasurer, Graham Lusk.

Thirty third meeting.

Cornell University Medical College, April 21, 1909. President Lee in the chair.

Members present: Atkinson, Auer, Burton-Opitz, Elser, Ewing, Flexner, Famulener, Gies, Janeway, Joseph, Kast, Lamar, Lee, Lewis, Lusk, Mandel (J. A.), Meltzer, Meyer, Morse, Noguchi, Norris, Oertel, Park, Pearce, Shaffer, Storey, Terry, Wallace, Wolf.

Members elected: William W. Hale, Andrew Hunter, H. S. Jennings, Peyton Rous, E. E. Southard, Charles R. Stockard, John L. Todd.

Thirty fourth meeting.

The Rockefeller Institute for Medical Research, May 26, 1909. President Lee in the chair.

Members present: Auer, Beebe, Ewing, Famulener, Flexner, Gies, Hatcher, Joseph, Lee, Lewis, Loeb (Leo), Morse, Meyer (Gustave), Pearce, Shaffer, Sherman, Terry, Van Slyke, Wallace, Weil, Wolf.

Members elected: J. W. Draper Maury, C. W. Edmunds, Adolph Meyer.

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WOODRUFF, LORANDE LOSS ..... Yale University.

YATSU, NAOHIDÉ ..... University of Japan.
YERKES, ROBERT M. ..... Harvard University.

ZINSSER, HANS ..... Columbia University.

Total number of members at the close of the academic year, 1908–'09: **185.**

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1903–1910.

	1903–'04	1904–'05	1905–'06	1906–'07	1907–'08	1908–'09	1909–'10
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Vice-President	Park	Ewing	Dunham	Dunham	Morgan	Morgan	Gies
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Treasurer	Calkins	Calkins	Calkins	Calkins	Calkins	Lusk	Lusk
Secretary	Gies	Gies	Gies	Gies	Gies	Gies	Opie

[1] Council — The Past Presidents and the Officers.

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Mt. Sinai Hospital.—Charles A. Elsberg.

New York City College.—Max Morse, Thomas A. Storey.

New York City Departments. Education.—C. Ward Crampton. *Health.*—James P. Atkinson, Edwin J. Banzhaf, L. W. Famulener.

New York Hospital.—Douglas Symmers.

New York Polyclinic Medical School.—Isaac Adler.

New York Post-Graduate Medical School.—Ludwig Kast.

New York University.—Harlow Brooks, Edward K. Dunham, Graham Lusk, Arthur R. Mandel, John A. Mandel, John R. Murlin, William H. Park, Richard M. Pearce, George B. Wallace.

Pathological Institute, Wards Island.—Adolph Meyer.

Rockefeller Institute for Medical Research.—John Auer, Alexis Carrel, Simon Flexner, Walter A. Jacobs, Don R. Joseph, Richard V. Lamar, P. A. Levene, Paul A. Lewis, S. J. Meltzer, Gustave M. Meyer, Hideyo Noguchi, Eugene L. Opie, Peyton Rous, B. T. Terry, Donald D. Van Slyke.

Sage Institute of Pathology, City Hospital.—Horst Oertel.

St. Francis Hospital.—Fritz Schwyzer.

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819 Madison Avenue.—H. D. Dakin.

449 E. 57th Street.—Isaac F. Harris.

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Albany Medical College.—Holmes C. Jackson.

Baltimore Medical College.—Charles E. Simon.

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OF THE

SCIENTIFIC PROCEEDINGS.

By **BERTHA I. BARKER.**

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PROCEEDINGS

OF THE

SOCIETY FOR

EXPERIMENTAL BIOLOGY AND MEDICINE

THIRTIETH MEETING

COLLEGE OF PHYSICIANS AND SURGEONS

COLUMBIA UNIVERSITY

NEW YORK CITY

OCTOBER 21, 1908

VOLUME VI

No. 1

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DECEMBER 1, 1908

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THIRTY FIRST MEETING

ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

NEW YORK CITY

DECEMBER 16, 1908

VOLUME VI

No. 2

NEW YORK

JANUARY 15, 1909

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NEW YORK UNIVERSITY AND BELLEVUE
HOSPITAL MEDICAL COLLEGE

NEW YORK CITY

FEBRUARY 17, 1909

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MARCH 15, 1909

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NEW YORK CITY

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No. 4

NEW YORK

1909

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THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

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MAY 26, 1909

VOLUME VI

No. 5

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1909

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St. Francis Hospital.—Fritz Schwyzer.

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Baltimore Medical College.—Charles E. Simon.

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Cooper Medical College (San Francisco).—William Ophüls.

Massachusetts Institute of Technology.—Percy G. Stiles.

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Wistar Institute of Anatomy (Philadelphia).—H. H. Donaldson, Shinkishi Hatai.

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Atkinson, Auer, Burton-Opitz, Elser, Ewing, Flexner, Famulener, Gies, Janeway, Joseph, Kast, Lee, Lamar, Lewis, Lusk, Mandel (J. A.), Meltzer, Meyer, Morse, Noguchi, Norris, Oertel, Park, Pearce, Shaffer, Storey, Terry, Wallace, Wolf.

Members elected at the thirty third meeting:

John L. Todd, Peyton Rous, H. S. Jennings, Andrew Hunter, Chas. R. Stockard, E. E. Southard, Wm. W. Hale.

Dates of the next two regular meetings:

May 26, 1909. October 20, 1909.

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Members present at the thirty second meeting:

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John F. Anderson, T. G. Brodie, L. J. Cole, Martin H. Fischer, Richard V. Lamar, Max Morse, Hans Zinsser.

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Dates of the next two regular meetings:

April 21, 1909. May 19, 1909.