FURTHER EXPERIMENTS ON THE EFFECTS OF LONG CONTINUED INTRAPERITONEAL INJECTIONS OF PROTEINS.

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In a previous article[1] we said: "Albumose (Witte's peptone) introduced parenterally into the guinea pig has very little, if any, harmful effect unless the oxidative powers of the organism are below normal. In view of the results of Longcope's experiments, as compared with ours, it seems possible that the more complex proteins will produce effects in the absence of decreased oxidation which the less complex ones will not produce under similar circumstances." These remarks were based upon a short series of experiments in which seven guinea pigs were given intraperitoneal injections of 1 per cent solution of Witte's peptone. Since the publication of this series we have continued the work, as will be detailed hereafter.

The different experiments are grouped as follows: 1. Guinea pigs which were given only Witte's peptone (Nos. 1, 2, 3, 4, 5, 18, 19). 2. Guinea pigs which received Witte's peptone, followed by chloroform anesthesia for fifteen minutes (Nos. 11, 12, 21, 22). 3. Guinea pigs which received chloroform only (Nos. 24, 27, 29). 4. Controls (Nos. 20, 23). 5. White rats which received intraperitoneal injections of a solution of albumen (Nos. 12, 13). 6. White rats which received intraperitoneal injections of a solution of albumen together with subcutaneous injections of chloroform in oil (Nos. 21, 22). 7. White rats which received intraperitoneal injections of casein (Nos. 14, 17). 8. White rat which received intraperitoneal injections of casein together with subcutaneous injec-

[1] Woolley, P. G., DeMar, A., and Clark, D., *Science*, 1914, N.S., xl, 789.

tions of chloroform in oil (No. 16). 9. Controls (Nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10).

In the protocols the expression albumose solution means one prepared according to the following formula and then sterilized:

Witte's peptone	1.0 gm.
Sodium chloride	0.5 gm.
Distilled water	100.0 gm.

The albumen solutions were made by dissolving 10 mg. of desiccated egg-albumen in 2 cc. of distilled water. The solutions were freshly prepared before each inoculation.

The chloroform solution was a 2 per cent solution in sterile olive oil.

The casein was given in the form of a solution made by dissolving 1 gm. of casein in 100 cc. of normal sodium hydrate and then carefully neutralizing (to phenolphthalein) with normal hydrochloric acid.

Group I.

Guinea Pig 1.—Weight 400 gm. This animal received 17 daily injections each of 1.5 cc. of the peptone solution, a total of 25.5 cc., or 0.255 gm. of albumose. It died suddenly on the day following the last injection. The cause of death was not discovered. The postmortem examination was done while the animal was still warm, and showed no other changes than a slight mediastinal edema, moderate hyperplasia of the lymph nodes, and congestion of the lungs, liver, spleen, and kidneys. Microscopic examination of the tissues showed edema and congestion with occasional small hemorrhages in the kidneys, with a few areas of small round cell infiltration, enormous congestion of the adrenals, edema and focal necroses of the thymus, and hyperplastic changes associated with congestion in the lymph glands and spleen. The spleen was more than normally pigmented.

Guinea Pig 2.—Weight about 350 gm. This animal received 57 daily injections each of 1.5 cc. of the peptone solution, a total of 85.5 cc., or 0.855 gm. of albumose. During the period of treatment it gave no sign of any untoward effects of the treatment. It ate well, lost no weight, and was finally chloroformed 72 hours after the last injection. The postmortem examination was done while the body was still warm. The organs showed no abnormal macroscopic or microscopic lesions, other than a moderate, generalized congestion associated with a very moderate edema of the parenchymatous organs. This, however, was no more than is usual after chloroform anesthesia.

Guinea Pig 3.—Weight about 400 gm. This animal received 30 daily intraperitoneal injections each of 1.5 cc. of the peptone solution, a total of 5 cc., or 0.45 gm. of albumose. It was killed with chloroform. The postmortem exami-

nation showed only a very moderate congestion and edema of the liver, spleen, kidneys, and adrenals, and a slight hyperplasia of the mesenteric lymph glands. Microscopic examination showed nothing abnormal except perhaps a slight degree of hyperplasia of the mesenteric lymph glands.

Guinea Pig 4.—Weight about 400 gm. This animal received 5 cc. of the peptone solution each day for 7 days, a total of 35 cc., or 0.35 gm. of albumose. It was killed with chloroform. At autopsy nothing abnormal was found. Microscopic examination was also negative.

Guinea Pig 5.—Weight about 350 gm. This animal was treated in the same manner as No. 4 for a period of 20 days, during which time it received a total of 100 cc. of the peptone solution, or 1 gm. of albumose. It was chloroformed and autopsied. During the period of treatment it lost 87 gm. in weight. At autopsy nothing noticeable was found except a partially healed meager exudate on the surface of the spleen. The peritoneal cavity contained 2 cc. of a clear fluid. Microscopic examination of the tissues showed no lesions except in the case of the spleen, in which there was an increased amount of pigment and a moderate hypertrophy of the Malpighian follicles. The capsule was thickened.

Guinea Pig 18.—Weight 470 gm. This animal received 5 cc. of the peptone solution, intraperitoneally, each day for 42 days, a total of 210 cc., or 2.1 gm. of albumose. At the time of death it weighed 485 gm., a gain of 15 gm. It was killed with chloroform. The organs showed but little change. The spleen was congested; the liver and kidneys showed occasional foci of small round cells. The peritoneal cavity contained no free fluid.

Guinea Pig 19.—Weight 452 gm. This animal received exactly the same treatment as Guinea Pig 18. At the end of the experiment the weight had dropped to 420 gm., a loss of 32 gm. The organs showed more noticeable microscopic changes than those of Guinea Pig 18. In the kidney the small round cell infiltration was rather diffuse,—the foci of accumulation were more numerous. The liver was unusually congested. The spleen was diffusely hyperplastic. The adrenal exhibited small round cell infiltration in foci in the medulla, and the cortex showed an unusual amount of lipoid metamorphosis. In the medulla there was an increase of hyaline material.

Remarks.—This group of experiments seems to show that comparatively large doses of albumose introduced in small doses over a considerable period of time have practically no harmful effect upon the organs of the body, unless it may be that the foci of small round cells are due to the treatment. It is possible that were the experiments continued over very long periods of time these foci might become fibroid.

Group II.

Guinea Pig 12.—Weight 412 gm. This animal received 50 intraperitoneal injections of 5 cc. of the peptone solution in the course of two months, a total of 250 cc., or 2.5 gm. of albumose. After each injection it was submitted to

deep chloroform anesthesia for 15 minutes. After 25 treatments the weight had increased to 455 gm. At the end of the treatments the weight was 485 gm. The postmortem examination revealed nothing macroscopically abnormal, and physically the animal seemed to be in good condition. Histological examination showed that there was a certain amount of anatomic modification of the tissues of some of the organs. The report was as follows: The kidney shows a well marked edema and cloudy swelling. The glomerular spaces are dilated and the tufts compressed, and in the spaces there is considerable coagulated albuminous material. About the glomeruli there are frequent small accumulations of small round cells, and in the outer layers of the cortex there are occasional lines of interstitial fibrosis. The whole organ showed congestion. The liver showed a very well developed edema, to the extent that in many areas the cells show what seem to be hydropic changes. With this is associated congestion and very moderate interstitial fibrosis as exemplified in the occasional collections of small round cells in the perilobular connective tissues. The spleen shows enormous hyperplasia of the Malpighian follicles together with some increased pigmentation. Within the corpuscles there is evidence of cellular fragmentation. The adrenals show a few collections of formative cells in both medulla and cortex, chiefly in the latter. The other organs revealed nothing remarkable.

Guinea Pig 11.—Weight 445 gm. This animal was treated in exactly the same way as No. 12. After 25 treatments it weighed 482 gm., and at the end of the experiment 565 gm. The report of the histologic examination stated that the changes were similar to those found in No. 12, except that there were a few retention cysts in the kidneys and that there was nothing of note in the adrenals except an intense congestion.[2]

Guinea Pig 21.—Weight 365 gm. This animal received 40 daily injections (intraperitoneal) each of 5 cc. of the albumose solution, and each injection was followed by 15 minutes of deep chloroform anesthesia. At the end of the experiment the weight had fallen to 340 gm., a loss of 25 gm. It was killed with chloroform. The cellular and organic changes were practically the same as those exhibited by Guinea Pig 19, though somewhat less in degree.

Guinea Pig 22.—Weight 340 gm. This animal was treated in exactly the same manner as Guinea Pig 21. At the end of the experiment it weighed 360 gm., a gain of 20 gm. The sections made from the organs showed changes similar to those described in Guinea Pig 18.

Remarks.—It was the results obtained in the first two animals in this group which, compared with those of Group I, called forth the remarks quoted in the first paragraph of this report. Chloroform undoubtedly reduces the oxidations of the body, and when administered at the same time with other substances may lead to the production of grave changes in the tissues, as Opie[3] has shown. It

[2] The histologic examinations in these cases were made by Dr. T. H. Kelly, who had no knowledge of the experimental procedures used in the individual cases. They were subsequently verified by one of us.

[3] Opie, E. L., *Tr. Assn. Am. Phys.*, 1910, xxv, 140.

is, however, to be remarked at this place that the results in the last two animals do not support the conclusion that because chloroform may do these things, it must do them. What conditions were present in Animals 11 and 12 to cause them to react differently, we cannot tell. They were from a different lot of animals from Nos. 21 and 22; they were fed more liberally with fresh vegetables than the latter, because they were procured in the late summer. But, on the other hand, they were of the same lot as Nos. 1, 2, 3, 4, and 5. Nos. 21 and 22 belonged in the same lot as 18 and 19. Still, perhaps the effect is dietary.

Group III.

Guinea Pig 24.—Weight 340 gm. This animal was treated on successive days by deep anesthetization for 15 minutes. At the end of the experiment it had lost 55 gm. It was killed by complete anesthetization. The tissues showed no changes other than congestion.

Guinea Pig 27.—Weight 616 gm. This animal was treated in exactly the same manner as was Guinea Pig 24, except that the number of anesthetizations was 9. After the 9th treatment it weighed 620 gm. The organs were similar to those of Guinea Pig 24.

Guinea Pig 29.—Weight 358 gm. This animal was treated like the two preceding, except that it received 40 treatments. At the end of the experiment it weighed 435 gm., a gain of 77 gm. The organs showed no macroscopic abnormality. Sections showed intense fatty metamorphosis of the liver and little else.

Remarks.—Apparently chloroform alone produces no more change, so far as interstitial changes are concerned, than the combination of chloroform and albumose. It does reduce oxidations and therefore tends to bring about an increase in weight, largely perhaps because of a decrease in oxidation of the fats.

Group IV.

Guinea Pigs 20 and 23.—These were normal controls, the organs of which were used for comparison. In each there were occasional fibrotic glomeruli in the kidney and occasional foci of small round cell infiltration.

Remarks.—The preceding group of experiments brings out no revolutionary fact. They merely indicate that while albumose administered, as we have done it, to normal guinea pigs produces, as a rule, no obvious symptoms or histologic changes, even when chloro-

form is given at the same time, yet occasionally early sclerotic changes appear.

Group V.

White Rat 12.—Weight 286 gm. This animal received 25 injections on successive days, each of 5 mg. of albumen. At the end of the experiment it weighed 305 gm. Postmortem examination showed no gross abnormality. Sections showed excessive edema and cloudy swelling, and in some of the glomerular spaces small mounts of coagulated albumen.

White Rat 13.—Weight 225 gm. This animal was treated in the same manner as Rat 12, except that it was given 44 injections, a total of 220 mg. of albumen. At the end of the experiment the weight had fallen 40 gm. The animal was killed by a blow on the neck.

The organs were apparently normal, macroscopically. The sections of the organs showed a considerable degree of cloudy swelling, especially of the convoluted tubules. In places the appearances were almost those of necrosis. Otherwise there were no microscopic changes, except a small granulomatous area in the liver.

Remarks.—The only essential difference in the tissues of Rats 12 and 13 lay in the presence of albumen in the glomerular spaces in No. 12, which was killed with chloroform. In No. 13 the marked cloudy swelling was perhaps due to whatever produced the granuloma.

Group VI.

White Rat 21.—Weight 282 gm. This animal received 40 daily injections of 5 mg. of albumen, intraperitoneally, and subcutaneous doses of 0.5 cc. of a 2 per cent chloroform solution in oil. At the end of the series it had lost 12 gm. in weight. Neither the organs nor the sections showed anything remarkable.

White Rat 22.—Weight 273 gm. This animal was treated for 7 days, like Rat 21. It lost no weight. The organs showed merely edema.

Group VII.

White Rat 14.—Weight 163 gm. This animal received 25 injections, on successive days, of 10 mg. of casein. At the end of the experiment it weighed 190 gm. Nothing anatomic or histologic seemed to result from the treatment.

White Rat 17.—Weight 290 gm. This animal received daily intraperitoneal injections of 10 mg. of casein for 17 days, followed by 23 similar injections each of 70 mg. of casein. At the end of the series it had lost 20 gm. There were no essential changes observed, macroscopically or microscopically, in the organs or tissues.

Group VIII.

White Rat 16.—Weight 310 gm. This animal was treated exactly like Rat 17, except during the last 23 days, when it was receiving 20 mg. of casein daily.

It also was given 0.5 cc. of the chloroform oil solution daily. At postmortem examination there was no evidence of gross changes, and sections showed nothing more than marked congestion and cloudy swelling.

Group IX.

White Rats 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.—These animals of various weights and ages furnished material for comparison.

Rat 1 had been fed on a strictly meat diet for several weeks.

Rat 2 had been fed on grain and vegetables for several weeks.

Rats 4 and 5 had been injected with trypan blue.

Rats 6, 7, 8, 9, and 10 had been given a single injection of uranium acetate, and had been killed after 24 to 48 hours.

DISCUSSION.

As stated in our earlier report, it was our object in carrying out this series of experiments to determine, if possible, the organic effects of certain substances of protein nature which might be produced within the tissues, or which might be absorbed from the intestinal tract.

Longcope[4] has said that parenteral digestion of egg-albumen may (under certain circumstances) seemingly produce organic renal and hepatic changes. This may be taken to mean that splitting of the whole protein (egg-albumen) leads to the production of these phenomena; that the effects are the results of the irritant action of substances set free during splitting; or that moieties of the protein molecule, by making abnormal combinations, may act as irritants or in some other way embarrass the functional cells of the tissues and at the same time stimulate the overgrowth of fibrous tissue.

Longcope produced the effects which he reported, not by the mere administration of albumen, but by so timing the injections that a mild state of anaphylaxis resulted. In our series we have not done this, but have endeavored to discover whether the substances which we have used would produce similar effects in the absence of anaphylaxis, and whether, at the same time, the addition of materials (such as chloroform) which hinder the oxidations in the cells, would modify or intensify the reactions. In a former series of experiments Woolley and Newburgh[5] showed that indol and tyrosine pro-

[4] Longcope, W. T., *Jour. Exper. Med.*, 1913, xviii, 678.

[5] Woolley, P. G., and Newburgh, L. H., *Jour. Am. Med. Assn.*, 1911, lvi, 1796. Newburgh and Woolley, *Lancet-Clinic*, 1912, N.S., lxviii, 404.

duced no remarkable changes. In this series we have shown that egg-albumen, casein, and albumen give practically the same negative results whether or not chloroform is administered with them.

In all our experiments we have used comparatively small doses (at any rate they were not massive); because if, as has been suspected, such anatomic conditions as chronic interstitial nephritis are the result of absorption from the gastro-intestinal tract, then the quantities of materials absorbed are probably small, and the process covers a considerable period of time. Perhaps we have not continued our work over a long enough period. However that may be, there is evidence that the living proteins in the form of bacteria are far more potent than the non-living, and that even the less pathogenic ones may produce greater damage in the length of time covered in our experiments than has been occasioned by the substances we have used.[6] These substances (microorganisms) are entering the system continuously, and since we know that they produce, often rapidly, anatomic changes, a series of experiments such as ours is chiefly of value in proving the relative lack of danger from absorption of the substances which we have employed,—at least in normal animals.

SUMMARY.

We have attempted to discover whether or not certain protein materials, such as albumose, casein, and albumen, when introduced parenterally (peritoneally) into experimental animals, are able to produce organic lesions. These proteins were used alone or in combination with chloroform, which was administered in oil and as an anesthetic. Our results were negative.

[6] Opie, *loc. cit.*