TABLE I
Ineffectiveness of following compounds in experimental poliomyelitis of monkeys.

| Compounds | No. Treated | Dose per Kilo* | No. Doses | Results |
|---|---|---|---|---|
| Hexamethylamine | 2 | .025 gm. | 8 | died |
| Metaphen | 2 | .0005 " | 8 | " |
| Mercurochrome | 1 | .005 " | 6 | " |
| Pregl's solution of iodine | 2 | .5 cc. | 8 | " |
| Sodium and gold thiosulphate | 1 | .0003 gm. | 7 | killed |
| Sodium ricinoleate | 2 | .005 " | 5 | " |
| Neoarsphenamin | 1 | .02 " | 8 | died |
| Neutral acriflavin | 1 | .010 " | 6 | " |
| Tryparsamide | 2 | .03 " | 6 | " |
| Bismuth Potass. Tartrate | 1 | .002 " | 5 | killed |

*By intravenous injection every 3 days; treatment begun upon first signs of poliomyelitis following intracerebral inoculation with virus 6 to 9 days previously.

In every instance paralysis progressed under treatment; 7 succumbed to the disease while the remaining 3 were chloroformed when it was apparent that treatment was ineffective.

## 6990 C

## Concerning the Absorption of Unsplit Protein Through the Lacteals.*

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It is commonly assumed that allergic responses to ingested foods are due to the passage of unchanged protein from the bowel into the blood. Alvarez, who had seen a few cases in which unfavorable reactions to food appeared to be worse when patients ate much fat with the offending protein, suggested to me that an unguarded pathway might be through the lacteals and thoracic duct into the blood stream. In this way the protein would escape possible change in the intestinal wall and in the liver. Somewhat in favor of this view is the reported observation that food reactions are less likely to occur if the patient first takes a dose of hydrocarbon oil; theoretically, it might block the lacteals.

A number of investigations, inspired by the contributions of Uhlenhuth,[1] and of Hamberger and Sperk,[2] attempted to identify

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1 Ascoli, M., and Vigano, L., *Z. f. physiol. Chem.*, 1903, **39**, 283.

unchanged protein in the blood of animals by means of the precipitin reaction, but unfortunately the results were conflicting. The studies of Hektoen, Kanai, and Dragstedt[3] may be mentioned in this regard. The difficulties in detecting foreign protein in the blood by precipitin methods are considerable, not only because the material is so greatly diluted, but, as has been frequently shown, because it leaves the blood stream so rapidly. The work of Walzer[4] and of Smyth and Stallings,[5] in which the newer principle of passive transfer in human beings was utilized, indicated that a detectable amount of certain proteins frequently enters the blood in unaltered form by way of the intestine. More recently, the experiments of Nedzel and Arnold[6] on the influence of egg-white on the absorption of bacteria from the intestinal tract, and the studies of McDaniels[7] on the effect of egg white in oral vaccination of mice against pneumococci, have given new impetus to studies in this field.

A survey of the literature shows that others in the past have searched for foreign protein in the lymph of the thoracic duct with the help of the precipitin reaction. Ascoli and Vigano[8] in studying such absorption noted the unexpected presence in rabbit serum of precipitin bodies for fasting dog lymph. This has been confirmed by others so that positive results in such experiments may be open to question. Recently, Ishikawa[9] reported his inability to detect unaltered egg albumin in the thoracic duct lymph of dogs after oral administration. He could find it only when he operated on the animals and injected the protein directly into a loop of intestine. Matsui and Iida are quoted by Ishikawa as having obtained results similar to his, but their papers are not accessible to me.

Since the results of precipitin tests can now be controlled by newer immunologic procedures, the problems of absorption of unsplit protein through the lacteals was subjected to further investigation. The present communication briefly summarizes the data obtained in this study.

1. *Preparation of anti-egg-albumin rabbit serum.* Eight rab-

[2] Coca, A. F., and Grove, E. F., *J. Immunol.*, 1925, **10**, 445.

[3] Hamberger, F., and Sperk, B., *Wien. klin. Wchnschr.*, 1904, **17**, 641.

[4] Hektoen, Ludwig, Kanai, P. H., and Dragstedt, L. R., *J. Am. Med. Assn.*, 1925, **84**, 114.

[5] Ishikawa, Issaku, *Jap. J. Med. Sc.*, IV. Pharmacology, 1928, **2**, 205.

[6] McDaniels, H. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 587.

[7] Nedzel, A. J., and Arnold, Lloyd, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 358.

[8] Smyth, F. S., and Stallings, M., *J. Allergy*, 1931, **3**, 16.

[9] Tomcsik, Joseph, and Kurotchkin, T. J., *J. Exp. Med.*, 1928, **47**, 379.

bits were given a series of alternating intravenous and subcutaneous injections of egg albumin at intervals of 4 days, starting with 1.5 cc. of a 1% solution in saline per kilo of body weight, and doubling the amount with each injection. Those rabbits the serum of which attained a precipitin titer of 1:80,000 to 1:100,000 were bled under sterile conditions and the serum was stored in the ice-box.

2. *Collection of lymph, technic of feeding.* Under ether anesthesia, the left internal jugular vein of fasting dogs, weighing 12 to 13 kg. was found with aseptic precautions, and all venous connections were ligated so that only the thoracic duct drained into it. A glass cannula was fastened in the vein and connected with a length of rubber tubing which was brought out to the surface through a stab wound. The original incision was closed in the usual manner. From 1½ to 2 hours after the animals had recovered from the operation samples of lymph were collected. With the animal resting, further specimens of lymph were obtained at intervals. Heparin was used as an anticoagulant. Later, food was given by stomach tube.

3. *Precipitin reactions.* The material to be tested (lymph from the fasting animal, physiologic saline controls, known and unknown concentrations of egg albumin in saline solution or lymph) was layered over anti-egg-albumin rabbit serum in minute test tubes 3 mm. in diameter and 30 mm. long. After standing for one hour at room temperature, a record of the condition at the surface of contact was made. It was determined by control experiments that the heparin did not interfere with the precipitin reaction.

4. *Technic of Tomcsik and Kurotchkin.*[10] As outlined by these investigators, guinea pigs weighing 250 to 280 gm. were passively immunized by the intraperitoneal injection of from 2 to 4 cc. of anti-egg-albumin rabbit serum. Twenty-four hours later, the intravenous administration of 1 cc. of 1 to 1,000 or 1 to 2,000 egg albumin solution (1 mg. to 0.5 mg.) to such animals invariably resulted in typical anaphylactic shock and death.

5. *Technic of Prausnitz and Kustner (atopic reagins).* By the method of Coca and Grove,[11] 0.1 cc. of anti-egg-albumin rabbit serum was injected intracutaneously in a series of rabbits, and after an interval of half an hour samples of material to be tested were similarly injected at the same sites. The animals were observed for 2 hours, and again at the end of 24 hours, for evidence of local reaction.

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[10] Uhlenhuth, *Deutsch. med. Wchnschr.*, 1900, **26**, 734.

[11] Walzer, Matthew, *J. Immunol.*, 1927, **14**, 143.

Unfortunately precipitin reactions between the immune rabbit serum and control samples of lymph from fasting dogs were common, and in only 4 experiments were such phenomena absent. Unaltered egg albumin in the thoracic duct lymph was not demonstrated. The simultaneous feeding of fat in one experiment did not cause a detectable amount of unsplit protein to appear in the lymph. Ascoli and Vigano attached significance to the fact that if lymph of fasting dogs gave faint precipitates with immune rabbit serum, the feeding of egg albumin produced heavier precipitation. This was true in our experience, but the feeding of 500 cc. of whole sheep blood caused similar effects, showing that the effect is not specific.

By means of anaphylactic reactions[9] absorption of unaltered protein through the lacteals could not be shown. All guinea pigs used in these tests were subsequently given intravenous injections of 1 cc. of 1:1,000 egg albumin in physiologic saline solution or in control lymph of fasting animals approximately one hour after the first intravenous injection, with death in every instance by anaphylactic shock.

Attempts to detect unaltered egg albumin by utilizing atopic reagins (method of Coca and Cole) were entirely unsuccessful.

A possible objection to the work may be that raw egg white is perhaps the least desirable protein to be used in such experiments, since it is often so poorly absorbed in the bowel of the dog.

*Conclusion.* In the dog, absorption of unaltered egg-albumin from the intestinal tract by way of the lacteals and thoracic duct could not be demonstrated.

## 6991 P

## A Test for the Demonstration of Estrin in the Blood of Women.*

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It was previously shown[1] that a mucification of the vaginal mucosa of adult spayed mice may readily be induced by the injection of blood serum from women in the premenstruum or in early preg-

* Supported in part by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine.