TABLE III.
Positive Serological Reactions of Patients Having Clinical Evidence of Toxoplasmosis.

Patient	Clinical manifestations	Test	
		Neutralization*	Complement-fixation (Titer)
A	Fatal case of toxoplasmosis in infant	Strongly pos.	1:32
B	Infant with chorioretinitis, cerebral calcification and mental retardation	Positive	1:128
C	Healthy mother of infant A	Strongly pos.	1:128
D	Healthy mother of infant B	Positive	1:128
E	Healthy mother of infant with congenital cataracts	,,	1:16
F	Adult with cutaneous nodules, chorio-retinitis and fever	,,	1:128

* Neutralization by the test serum of a 1:100 dilution of toxoplasma-infected tissue when the mixture is injected intracutaneously in the rabbit is regarded as a positive reaction; neutralization of a 1:20 dilution as a strongly positive.

blood, and sternal marrow gave negative results. Because of these serological findings, the patient was tested for cutaneous reactivity to toxoplasma material. For this purpose, 1/100 and 1/1000 dilutions of standard complement-fixing antigen and a 1/1000 dilution of normal egg antigen prepared in a similar fashion were injected intracutaneously in 0.1 cc amounts. Although there was no immediate reaction at any of the sites, both of the areas receiving the toxoplasma antigen developed erythema and induration at 24 hours which persisted for several days; the lesion at the site of the 1/100 dilution was 1½ x 2 x 0.1 cm and at the 1/1000 dilution was 1 x 1 x 0.1 cm. No reaction was elicited at the site of the injection of normal egg material.†

Summary. Inoculation of embryonated eggs with toxoplasma resulted in a generalized parasitic disease of this host with a fatal termination. The agent could be maintained by serial passage of embryo brain. Chorioallantoic membranes from infected eggs contained a stable, specific soluble antigen which fixed complement with toxoplasma immune animal serum. Human sera from both proven and suspected cases of toxoplasmosis which were known to contain neutralizing antibody against the parasite also fixed complement with chorioallantoic membrane antigen.

† These studies were performed in collaboration with Drs. A. J. Brennan and T. Brown, Mount Alto Hospital, Washington, D.C., and will be reported in detail elsewhere.

16214

Influence of Oxophenarsine on Hypoglycemic Action of Insulin.

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Noting the results obtained by Barron and Singer[1] in inhibiting the activity of certain enzymes with arsenoxides and their use of this reaction as a test of the essential SH groups of such enzymes, the author thought it of interest to incubate insulin under sterile conditions with the arsenoxide, oxophenarsine (3-amino-4-hydroxyphenyl arsenoxide hydrochloride). While enzyme workers assume that reaction is only with free SH groups, it seemed possible that a part of the arsenoxide might reduce the S-S linkages of insulin to –SH,

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1 Barron, E. S. G., and Singer, T. P., *Science*, 1943, **97**, 356.

being converted to the arsonic acid, and that then another portion might react with insulin –SH to form thioarsenites. It was expected that such a reaction would cause loss of insulin potency. Under the proper conditions rather a prolongation of action but not a loss of potency was observed.

Methods. The insulin used was Lilly Isletin, 20 units/cc. The oxophenarsine hydrochloride was pure 3-amino-4-hydroxyphenyl arsenoxide hydrochloride hemialcoholate† (mol. wt. 254.4), and not the commercial Mapharsen, containing sucrose and sodium bicarbonate buffers.

The insulin bioassay method in preliminary tests was the 5-hour rabbit blood sugar method of Marks.[2] In later tests by the Eli Lilly Co. their standard method of 5-6-hour rabbit blood sugar test was used (tests at 1.5, 3, 5, and 6 hours).

Incubation of insulin and oxophenarsine: Into sterile rubber capped test tubes or bottles under sterile conditions a given volume of the insulin (*e. g.*, 10 cc of 20 units/cc) was delivered and an equal volume of sterile n/320 HCl in 0.85% saline was added. After replacement of the air with nitrogen gas the oxophenarsine salt in proper amount was dropped in, the container sterilely capped, shaken, and incubated for 18 hours at 37°C. The fluid was then used for the dilution (with the same sterile acid saline) to the 1 or ½ unit per 0.5 cc dose injected into the rabbits. Control animals received the same insulin, which in the 1-1 dilution with acid saline was incubated in similar manner without addition of the arsenical (hereinafter called the regular insulin).

Calculation of the molar equivalent of oxophenarsine per molar sulfur content of the insulin: Assuming that 20 units of insulin equalled 2.32 mg of insulin and taking Svedberg's value for the mol. wt. of insulin at 37,000 and the sulfur content at 3.2%, it was calculated that the molecule of insulin contained 37 atoms of sulfur and that a mole of oxophenarsine would be needed for each. (It may be noted that Stern and White's[3] claim of 36 atoms of sulfur, viz., 18 S-S links, would allow for a slight excess of oxophenarsine, while a higher mol. wt. with the same S percentage content would also allow an excess.) By calculation, with an equivalence of one mole of oxophenarsine per 37 atoms of sulfur, 1 mg of insulin would require 0.2534 mg of oxophenarsine salt, and 1 ml of 20 units/ml insulin would require 2.32×0.2534 or 0.5878 mg of the arsenical. Ten ml of 20 units/ml insulin would then need 5.89 mg for one mole, 11.78 mg for 2 moles. To allow a slight excess the above values were rounded to 6.00 mg and 12.00 mg respectively per 10 ml of 20 units/ml insulin.

Results. Preliminary work. An initial test by the Marks method, upon addition of 6.00 mg of oxophenarsine salt per 10 ml of 20 units/ml insulin and incubation, showed an apparent loss of potency from 100% for the regular insulin to 87% for the arsenically treated insulin.

In the expectation of a possible further loss of potency with a doubled quantity of the arsenical, that amount was tried and the incubate tested on 5 rabbits, which, on the first day received the regular insulin (1 unit) and 2 days later received the unknown "treated insulin" (1 unit). This statistically invalid test showed an apparent increase in potency such as might be found in working in the upper flat part of the dose response curve. Hence, another set of 5 rabbits was tested, using ½ unit insulin doses, and again an apparent increase in potency was observed. At this point, however, the 6th hour blood sugars were taken and were found to average 26.1% below the normal at this time.

With the possibility that a delayed reaction insulin had been obtained, arrangements were made with Edward D. Campbell‡ of the Eli Lilly Co. to prepare similar batches of control insulin and oxophenarsin-treated insulin and make a statistically valid test by their standard method.

Results of the Lilly Laboratories test: Based on results obtained from 30 rabbits on

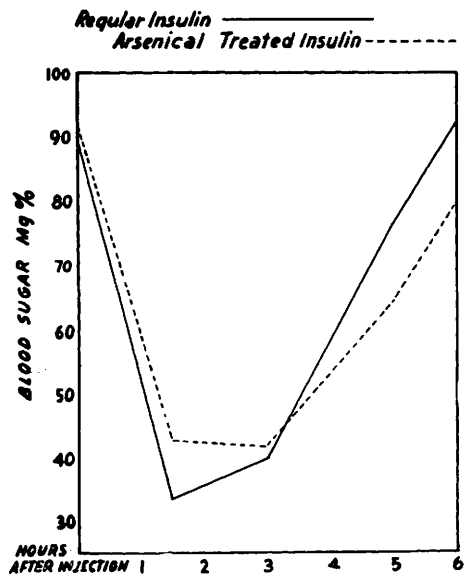
† Kindly supplied by Dr. Oliver Kamm of the Parke Davis Company.

2 Described in Burn, J. H., *Biological Standardizations, London,* 1938, p. 79ff.

3 Stern, K. G., and White, A., *J. Biol. Chem.,* 1937, **117**, 95; 1937, **119**, 215.

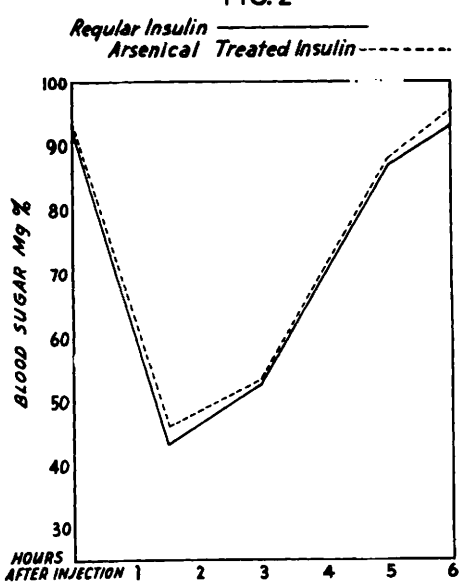
‡ We desire to thank Dr. G. H. A. Clowes and Dr. Campbell for arranging these tests.

FIG. 1



Comparison of the lowering of blood sugar level by oxophenarsine-treated insulin with the lowering induced by regular insulin at the same unit dose. Test conducted immediately after preparation. Avg mg % blood sugar of 30 rabbits for each curve.

FIG. 2



Repetition of the comparison after the arsenical-treated insulin had remained two weeks in the ice-box. Avg mg % blood sugar of 12 rabbits for each curve.

each test (control and unknown) the blood sugar curve shown in Fig. 1 was obtained.

There was no difference in the 2 insulins at the 3rd hour, hence there was no potentiation, but the marked differences at the 5th and 6th hours were typical of an insulin with a delayed reaction.

To test the stability of the As-insulin complex the two specimens were again compared after standing in the icebox for 2 weeks. Two groups of 12 rabbits each were used. The blood sugar curve shown in Fig. 2 was obtained.

The arsenical combination was unstable and at 2 weeks standing in the cold the insulin had regained the hypoglycemic properties of regular insulin.

Discussion. Despite the instability of the oxophenarsine-insulin compound the evidence of interaction may be of interest to those studying the mechanism of action of insulin. It raises the question whether an arsenical interaction with S-S linkages has occurred, such as thioarsenite bridge formation, to delay the normal destruction of insulin by glutathione as postulated by DuVigneaud,[4] or whether interaction might be with free amino groups of the hormone. Hallas-Moeller[5] has reported that reaction of insulin with phenyl-isocyanate produced a delayed reaction insulin claimed to form by a reaction of the isocyanate compound with the histidine and lysine free amino groups. But Stern and White[6] have claimed that acetylation of the free amino groups does not appreciably affect insulin activity, though acetylation of the tyrosine OH groups does. Whatever the mode of reaction may be, the compound formed is easily hydrolysed with regeneration of insulin. A further possibility is a brief, temporary partial inactivation of the insulin. At body temperature this effect may have been decreasing by the third hour, but be sufficient to produce

[4] (a) du Vigneaud, V., Fitch, A., Pekarek, E., and Lockwood, W. W., *J. Biol. Chem.*, 1931, **94**, 233; (b) du Vigneaud, V., *Cold Spring Harbor Symposia Quant. Biol.*, 1938, **6**, 275.

[5] Hallas-Moeller, K., Dissertation, Copenhagen, 1945; *via* author's summary, *Arch. Pharm. og Chemie*, 1945, **52**, 627.

[6] Stern, K. G., and White, A., *J. Biol. Chem.*, 1936, **122**, 371.

a delayed reaction.

Summary. On incubating insulin under sterile conditions in acid saline with 2 moles of oxophenarsine (3-amino-4-hydroxyphenylarsenoxide hydrochloride) for each atom of sulfur contained in the insulin, it was found (by blood sugar determinations) that the effect of the treatment was to prolong the action on the blood sugar beyond that of untreated insulin. However, after 2 weeks standing this delayed action effect had disappeared and the material gave a blood sugar curve identical to that of the control insulin. Possible mechanisms of the interaction of oxophenarsine and insulin are briefly discussed.

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Failure to Relate Hyaluronic Acid to Elevated Erythrocyte Sedimentation Rate in Rheumatic Diseases.*

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It has been demonstrated that many asymmetric macromolecular substances are capable of increasing the erythrocyte sedimentation rate (E.S.R.) when added to normal blood. The substances listed by Fahraeus[1] as having such an action were gelatin, agar, gum arabic, gastric mucin, fibrinogen, globulin, and sodium caseinate. In recent years, pneumococcus capsular polysaccharides,[2] desoxyribonucleic acid,[3,4] and hyaluronic acid[4] have been shown to exert a similar effect. Meyer reported[4,5] that a testicular extract containing hyaluronidase, an enzyme complex which depolymerizes hyaluronic acid, when added to the blood of rheumatic fever patients decreased the elevated E.S.R. to normal limits. This report seemed of significance since hyaluronic acid is believed to be a constituent of the amorphous ground substance of the connective tissue, the site primarily involved in rheumatic fever.[6]

The investigation reported here was undertaken to determine whether hyaluronic acid is present in the blood of patients with rheumatic diseases and whether this substance is responsible for the elevated E.S.R. commonly observed.

Materials. The sodium salt of hyaluronic acid was prepared by a modification of the method of Seastone.[7] An aqueous extract of umbilical cord was deproteinized by stirring with a chloroform-amyl alcohol mixture in a Waring blender. The polysaccharide was finally precipitated by the addition of 2 volumes of ethyl alcohol saturated with sodium acetate in the cold. Sodium hyaluronate prepared in this manner produced a clear or opalescent, highly viscous solution at a concentration of 1.0% To avoid discrepancies due to variations in the preparation, one batch was made and used throughout the experiment.

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‡ The authors wish to thank Drs. W. D. Robinson and W. D. Block of the Rackham Arthritis Research Unit for furnishing blood from cases of rheumatoid arthritis and salicylate levels used in this report.

1 Fahraeus, R., *Acta Med. Scandinav.*, 1921, **55**, 1.

2 Nungester, W. J., and Klein, L. F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 315.

3 Mori, S., *Kekkoku*, 1941, **19**, 50.

4 Meyer, K., Hahnel, E., and Feiner, R. R., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 36.

5 Meyer, K., Chapter 18, *Currents in Biochemical Research*, ed. by Green, D. E., 1946.

6 Klinge, F., *Ergeb. d. allg. Path. u. Path. Anat.*, 1933, **27**, 1.

7 Seastone, C. V., *J. Exp. Med.*, 1939, **70**, 361.