In this small series, the decrease in free cholesterol with incubation was usually less than normal with disease of the liver parenchyma and occasionally with extra-hepatic obstructive jaundice. The decrease in free cholesterol apparently did not parallel changes in serum bilirubin, alkaline phosphatase, serum albumin, or the cephalin flocculation test. There appeared to be some correlation with the percentage of cholesterol esters in the control serum.

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17281. Effect of Antihistamine on the Localization of Trypan Blue in Xylene Treated Areas of Skin.

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Some data and experimental observations have suggested that histamine may play a fundamental role in the development of local areas of inflammation.[1-3] In support of this, it has been shown that trypan blue following an intravenous injection localizes and concentrates in areas of skin previously injected with histamine.[4] The effects of antihistamine drugs on capillary permeability have been reviewed recently by Last and Loew.[5] These investigators found that the localization of trypan blue in areas of skin previously injected with different chemical and biological preparations was not modified by the intravenous injection of the antihistamine preparation "Benadryl".[5]

The present experiments were performed to study the effect of two of the more recent antihistamine preparations on the hyperemia and the localization and concentration of trypan blue in areas of skin treated with xylene.

Methods and materials. Fifteen rabbits were used. Their hair was carefully removed 24 to 48 hours preceding the time of the experiment. Two antihistamine preparations were used: Pyrrlidineethyl-phenothiazine hydrochloride (Pyrrolazote*); and, N,N-Dimethylly-N′ (alpha-pyridyl)-N′ (alpha-thenyl)-ethylenediamine hydrochloride (Thenylene†). A solution of each containing 10 mg per cc was made in physiologic sodium chloride. The injections of Pyrrolazote were made intravenously, while Thenylene was injected both intravenously and intraperitoneally. Ten cc of a 0.2% solution of trypan blue was injected intravenously. Xylene was carefully applied with a cotton applicator to local areas of skin at intervals varying from one to 140 minutes during the time that the rabbits were under the influence of the antihistamine preparations and before the dye was injected intravenously. The xylene treated areas were carefully observed during the development of hyperemia and during the time of the localization and concentration of the dye. These observations extended over a period of 2 hours.

In 8 rabbits 0.2 cc of the antihistamine preparations was injected intradermally from immediately to 60 minutes preceding the time of the intravenous injection of trypan blue. An equal volume of both a physiologic sodium chloride solution and distilled water was used as controls.

1 Lewis, Sir Thomas, London, Shaw and Shaw, 1927.

2 Findlay, G. M., *J. Path. and Bact.*, 1928, **31**, 633.

3 Mayer, R. L., *Ann. Allergy*, 1947, **5**, 113.

4 Rigdon, R. H., *J. Lab. and Clin. Med.*, 1942, **27**, 1554.

5 Last, M. R., and Loew, E. R., *J. Pharm. and Exp. Therap.*, 1947, **89**, 81.

* Obtained from the Upjohn Company, Kalamazoo, Mich.

† Obtained from the Abbot Research Laboratories, Chicago, Ill.

Experimental. Four rabbits were given Pyrrolazote intravenously. One received 23.31 mg/kg in 5 injections during a period of 85 minutes; a second, 24.21 mg/kg in 4 injections during a period of 48 minutes; a third, 15.54 mg/kg in 3 injections during a period of 137 minutes and the fourth, 16.5 mg/kg in 2 injections during a period of 16 minutes.

The areas of skin where xylene was applied became hyperemic within a minute and progressively increased in intensity for approximately 5 minutes, after which the reaction remained more or less constant for several hours then regressed. The rate of development and the intensity of the hyperemia that occurred following the application of xylene did not vary from that in rabbits similarly treated, but not given any antihistamine as previously reported.[6] Trypan blue likewise localized and concentrated in these xylene treated areas in a manner similar to that observed in rabbits not given any antihistamine.

All the xylene treated areas of skin were hyperemic. Trypan blue, however, localized and concentrated first in the last 2 areas where xylene was applied the shortest interval before the dye was given.

Three rabbits were given Thenylene both intravenously and intraperitoneally. One was given 28.11 mg/kg in 6 injections over a period of 37 minutes, another 16.64 mg/kg in 4 injections over a period of 35 minutes, and one, 17.57 mg/kg in 4 injections during a period of 40 minutes.

The areas of skin where xylene was applied were identical in these rabbits with those observed in animals given the Pyrrolazote and trypan blue. These 3 rabbits showed toxic symptoms produced by this antihistamine preparation during the time of the experiment.

Trypan blue localized and concentrated in each skin area of the 8 rabbits where both antihistamine drugs were injected intradermally. In 2 rabbits injected with Thenylene there was less dye after 30 minutes in the areas injected 35 minutes before the intravenous injection than in the areas injected 15 minutes and immediately preceding the intravenous injection of the dye. Trypan blue did not concentrate in any of the areas of skin previously injected with physiologic sodium chloride. Twenty minutes following the intravenous injection of trypan blue there was a small area at the site of the injection of the antihistamine preparation that was pale in color and did not stain blue. There was a zone of dye around this anemic area and, peripheral to it, there was a zone of edema which did not stain any deeper with trypan blue than surrounding normal skin. There were no significant changes in the manner of localization of trypan blue in the injected areas of skin after the first 30 minutes of the experiment.

Discussion. In these observations the two antihistamine preparations, Thenylene and Pyrrolazote, in the concentrations used apparently do not effect the development of hyperemia and the localization and concentration of trypan blue in xylene treated areas of skin. In previous studies it has been emphasized that the localization and concentration of trypan blue in areas of inflammation are not determined by the presence of hyperemia.[6] In support of this observation, Last and Loew have found that acetyl-B-methyl-choline (Mecholyl), although producing vasodilation, did not cause a trypan blue reaction.[5] Can it be that a local area of skin treated with xylene or injected with horse serum stains blue primarily as a result of a variation in the absorptive ability of the tissue cells, and not because there is only a change in the permeability of the capillaries? The vital staining of cells has been regarded by some as due to absorption of dye molecules by cell granules.[7]

According to Last and Loew,[5] appropriate concentrations of intradermally injected horse serum, tetracaine, codeine and heparin cause positive trypan blue reactions which are not modified by Benadryl. Arginin, which is a specific antagonist against some of the effects of histamine, does not prevent the production of a trypan blue reaction by either histamine or agents which liberate histamine.[8] The above observations are different from those

[6] Rigdon, R. H., *Arch. Surg.*, 1940, **41**, 101.

[7] Nagao, K., *J. Inf. Dis.*, 1921, **28**, 294.

[8] Rocha e Silva, M., and Dragstedt, C. A., *J. Pharm. and Exp. Therap.*, 1941, **73**, 405.

of Mayer who found that "pyribenzamine, a substance exhibiting strong and specific antihistamine properties, exerts a definite activity in experimental dermatitis".[3]

The localization and concentration of trypan blue following an intravenous injection, in areas of skin injected intradermally with antihistamine emphasizes the fact that substances other than histamine may cause a localization of this dye.

Summary The hyperemia that follows the local application of xylene apparently is not modified by the intravenous and intraperitoneal injections of the antihistamine preparations, Pyrrolazote and Thenylene. Likewise, the localization and concentration of trypan blue in the xylene treated areas of skin are not affected by these preparations of antihistamine. Trypan blue also localizes and concentrates in areas of skin injected intradermally with these preparations of antihistamine.

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17282. Studies on Elimination of Penicillin G in Dogs.*

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Rammelkamp and Keefer[1] found that only about 60% of intravenously administered penicillin could be recovered from the urine of man. This finding has been confirmed by later reports, although the recovered amounts vary from 40 to 99%.[2]

This paper deals with the mechanism of the elimination of penicillin in bilaterally nephrectomized dogs.

Methods. Healthy mongrel dogs weighing from about 6 to 12 kg were used. Crystalline penicillin G§ was injected intravenously in all experiments; the blood samples were obtained from another vein and the serum penicillin assayed against Staphylococcus 209 by a serial dilution technic.

Diffusion of Penicillin from Blood to Tissues. Results. A dog which had been bilaterally nephrectomized and the cystic and common bile ducts ligated was given an intravenous injection of 25,000 u/kg of crystalline penicillin G. The penicillin concentration decreased rapidly during the first hour but very slowly thereafter (Fig. 1). It is assumed that this initial decrease was due to

The diffusion of penicillin from blood to tissues

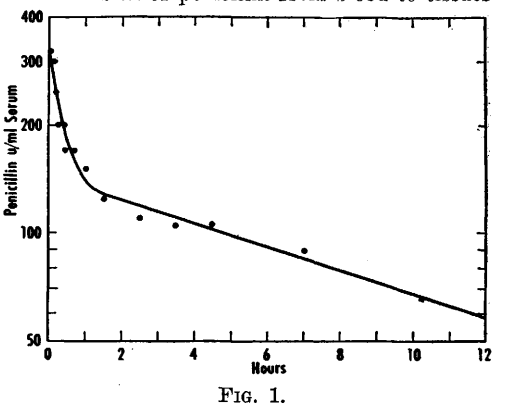

FIG. 1.

The serum penicillin concentration as a function of the time after a single intravenous injection. The inactivation has been partially blocked by bilateral nephrectomy and ligation of the cystic and common bile ducts.

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† Douglas Smith Foundation Fellow in Medicine.

‡ Research Fellow, U. S. Public Health Service.

1 Rammelkamp, C. H., and Keefer, C. S., *J. Clin. Invest.*, 1943, **22**, 425.

2 Herrell, W. E., Penicillin and Other Antibiotic Agents, W. B. Saunders Company, 1945.

§ The crystalline penicillin was supplied by Abbott Laboratories, Commercial Solvents Corporation, Lederle Laboratories, and Schenley Laboratories, Inc.