in all groups; microscopic examination revealed, in the cholesterol-fed animals, a moderate cholesterol deposition, diffuse in the hyperthyroid rats, and focal in the thiouracil fed animals. Isolated cases of early atheromata and widespread fatty infiltration were observed in the rats fed thyroid and cholesterol, and in both groups treated with thiouracil.

Received March 13, 1950. P.S.E.B.M., 1950, v73.

A Skin Test for Infectious Hepatitis.* (17758)

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Evidence has been obtained that the virus of infectious hepatitis (IH) can be propagated in minced chick embryo tissue culture and in the embryonated hen's egg(1,2). Virus thus cultivated induced mild hepatitis without jaundice in human volunteers but no laboratory test for the presence of virus has evolved as yet. However, as will be shown in this paper, amniotic fluid of chick embryos infected with IH virus may serve as skin test antigen with apparently specific results.

The Akiba strain of IH virus was passed 10 times through chick embryo tissue culture and then transferred in 0.2 ml amounts to the amniotic cavity of 8 day old chick embryos. After incubation at 37° for 7 days the whole contents of the eggs minus the albumen were emulsified in an approximately equal volume of saline by means of a Waring blendor and passed amniotically to fresh embryos. After 3 such passages, when slight liver damage became apparent in some of the embryos, only amniotic fluids were harvested and passed in further transfers and the incubation period was reduced to 5 days. Amniotic fluids of the 6th and 7th consecutive passage were used for IH skin test antigens. Control materials were prepared under identical conditions except for the fact that the amniotic series was initiated with buffered saline instead of the Akiba tissue culture material. The specific and control antigens were irradiated with ultraviolet light for a period 5 times that required to inactivate influenza virus. The materials were cultured aerobically and anaerobically, and tested for safety by intraperitoneal and intracerebral inoculation of mice and by intradermal injection of human volunteers with no history of IH. The IH and control antigens were stored at 4°C until used. They were administered in 0.1 ml amounts on the right and left forearm, respectively.

Results of 161 skin tests are shown in the Table. They were performed (a) in human volunteers in corrective institution No. 1 who, 3 months to 2 years previously had submitted to infection with IH virus as contained in stools and serum taken during the acute stage of IH (Akiba or NL Strain) or in tissue culture; (b) on volunteers in institution No. 2 injected 5 months to 5 years previously with the virus of serum hepatitis (SH); (c) on 62 random subjects of institution No. 1; (d) on 9 young children 2½ to 4 years old; and, finally, (e) on 10 cases of spontaneous IH 12 days to 6 months after onset of illness. Positive skin tests to IH were characterized by the development within 24 hours of an area of erythema of a somewhat brownish red tinge and of induration which as a rule exceeded 10 mm in diameter (4 to 45 mm) and which persisted, although decreasing in intensity, some-

* This investigation was conducted under the Commission on Liver Diseases, Armed Forces Epidemiological Board, Preventive Medicine Service, Office of the Surgeon General, U.S. Army.

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TABLE I.
Result of IH Skin Tests in Individuals with Past Histories of IH or SH and in Random Groups.

History of group	No. in group	No. showing negative control test	IH test	
			No. positive	% positive
Exposed to IH (I.* No. 1) Signs of illness	25	24	24	100
Exposed to IH (I. No. 1) No signs of illness	9	8	7	87.5
Exposed to SH (I. No. 2) Signs of illness	16	13	3	23
Exposed to SH (I. No. 2) No signs of illness	32	31	13	42
No exposure (I. No. 1)	62	56	23	41
No exposure (children)	9	9	1	11
Natural IH	10	10	10	100

* I = Institution.

times up to a period of one week. The control antigen caused no reactions in the skin in 149 of the cases reported here. However, in 12 of the individuals there were areas of pink erythema with little or no induration, measuring from 2 to 30 mm in diameter in 24 hours. These had, however, faded by the 2nd day in the majority of cases. The specific reactions in most instances appeared clearly separable from the non-specific ones by a difference in coloration, by the absence of marked induration and, frequently, by the size of the areas involved. However, for the time being, these cases have been omitted from the analysis of the results shown in the Table. It is felt at present that the optimal time for reading falls between 24 and 36 hours after the administration.

As can be seen in the Table, the skin test was strongly or weakly positive in all the volunteers previously exposed to IH virus who had shown clinical or laboratory signs of disease and also in 8 out of 9 of those who had failed to develop signs of infection, presumably because they were immune at the time of exposure. The one negative skin test was obtained in an individual who had been given a 10^{-6} dilution of IH serum containing possibly too little or no virus. In volunteers previously injected with SH virus the incidence of positive skin tests was much lower, regardless of whether or not they had revealed clinical or laboratory signs of disease. Furthermore, the incidence of positive skin tests in a random group of adult volunteers at institution No. 1, previously not experimentally exposed to either IH or SH viruses, was similar to that encountered in the SH series at institution No. 2. On the other hand, only 1 out of the 9 children without a history of recognized hepatitis gave positive tests. On further investigation this child was found to have suffered 6 months previously of an illness clinically compatible with hepatitis without jaundice at an institution where IH was prevalent. Each of the 10 patients convalescent of a recent attack of spontaneous IH showed positive skin tests.

These encouraging results are indicative of a specific test in that a difference in response of IH and SH patients is apparent. It is obvious, however, that the studies have to be enlarged and extended to other conditions of liver illness and other infectious diseases. Such studies are in progress.

The fact that approximately 39% of the adult individuals in the SH and control series gave positive tests with IH antigen may be an indication of past unrecognized infection with this agent. In the absence of reliable histories this point cannot be ascertained. However, that the positive skin test possibly denotes

immunity and the negative results susceptibility may perhaps be gleaned from a comparison of the incidence of experimental IH in the groups of volunteers and the results of the skin test in a similar group chosen at random in institution No. 1. Over a period of 2 years 54 volunteers were exposed and 34 developed clinical or laboratory signs of hepatitis, 8 with jaundice; *i.e.*, 63% were definitely susceptible. Of individuals with specific skin test responses, 33 or 59% were negative and presumably susceptible. Although this appears to be a striking agreement this point requires confirmation by studying the effect of natural or experimental exposure to IH virus in individuals with negative and positive skin tests, respectively.

Summary. A skin test antigen for infectious hepatitis (IH) has been prepared from amniotic fluid of chick embryos infected with IH virus. Thus far, it has given apparently specifically positive skin reactions in all the individuals with past histories of spontaneous or induced IH tested. In contrast, the incidence of positive tests among cases of past serum hepatitis was no greater than that found among an adult group chosen at random.

Received March 10, 1950. P.S.E.B.M., 1950, v73.

In Vitro Histamine Release from Sensitized Rabbit Blood Cells. Evidence Against Participation of Fibrinolysin. (17759)

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A considerable amount of circumstantial evidence has been presented to support the view that the release of histamine associated with anaphylaxis depends upon a proteolytic mechanism. The evidence has been obtained from studies in the dog, guinea pig, and rabbit and some of the studies appear to implicate the plasma protease, fibrinolysin (1-7). The evidence cited is indirect and circumstantial and a more direct type of evidence is desirable.

In this paper are presented the results of our studies on the question of whether fibrinolysin participates in the release of histamine from the blood cells of the sensitized rabbit by antigen, *in vitro*. The availability of (a) crystalline soy bean trypsin inhibitor(8), which is a potent inhibitor of fibrinolysin, and (b) purified bovine fibrinolysin and antifibrinolysin(9,10) offer a direct experimental approach to this problem.

Experimental. The details of rabbit sensitization, exsanguination, the *in-vitro* histamine release reaction, and histamine determination were presented previously(11). Silicone-coated glassware was used for the handling of whole blood and for the histamine release reaction. The authors are grateful to Dr. M. Kunitz for samples of crystalline trypsin and soy bean trypsin inhibitor, STI, and to Eugene C. Loomis for samples of purified bovine fibrinolysin and antifibrinolysin. Some of the STI used was prepared in our own laboratory by the method of Kunitz.

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