the operation and behave like normal rats that had been subjected to the same operation of partial hepatectomy and injected with saline.

Tolerant rats were then subjected to "functional" hepatectomy by evisceration according to Russel.[11] The operation is performed under ether anesthesia and lasts about 10 minutes. Half an hour after the operation the rats walk around and appear quite "normal." These operated rats also tolerate the maximum dose of morphine and behave like normal operated rats injected with saline. That such asphyxiated livers are effectively cut off from circulation is shown by the fact that the blood sugar falls. However, the rate of fall of blood sugar varies greatly from one animal to the other. The survival time of such animals treated with saline injections varies from 3 to 20 hours. In accordance with the observation of Ingle and Sheppard[12] we observed no clear correlation between the time of survival and the level of blood glucose at death.

It is difficult to interpret these results in any other way than to mean that tolerance is not due to metabolism of the morphine in the liver, spleen or gastrointestinal tract.

[11] Russel, *Am. J. Physiol.*, 1942, **138**, 95.

[12] Ingle and Sheppard, *Endocrinology*, 1945, **37**, 377.

16451

Organ Antigen in the Early Chick Embryo.

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It was previously shown[1] that early embryonic stages of the chick contain one or more antigens occurring in the adult serum. In the present report evidence is presented for the presence in the early chick of a second type of antigenic substance(s) which is common to several adult organs and is serologically distinguishable from the antigens of blood and yolk.

Our previous work on the chick[1] indicated that the serum-antigen(s) in the early stages was exclusively of vitelloid nature, that is, serologically similar to certain constituents of the yolk. Non-vitelloid constituents of the serum appear in later stages of development. In attempting to demonstrate a new organ-antigen in the early embryo, it is therefore necessary to show that it can be distinguished from both blood and yolk antigens, since one group of antigens might be common to a given organ and blood, whereas another group might be common to blood and yolk.

Preparation of antisera. Brains were obtained from chicks near the time of hatching (19-20 days of incubation). The chicks were perfused with 0.85% NaCl solution injected into the left ventricle and allowed to flow from the cut jugular veins until clear of blood. The roof of the cranium was cut away, the brain removed to sterile Petri dishes and frozen over dry ice. When properly perfused the brain is of a chalky-white color. Accumulated brains were crushed while frozen and dried *in vacuo* at −10°C. The dried tissue, very porous and friable, was broken up with a sterile pestle and mortar and stored in tightly-stoppered bottles at −25°C.

The brain powder was stirred into ice-cold saline in proportion of 100 mg of dry powder for every 3 ml of saline. After mechanical stirring for about an hour at 0° to 3°C, the suspension was left overnight at 3° to 5°C, and then centrifuged at 3000 R.P.M.

[1] Schechtman, A. M., *J. Exp. Zool.*, 1947, **105**, 329.

TABLE I.
Ring (Precipitin) Tests With Anti-brain Sera Layered Under Extracts of Blood and Organs of the 19 to 20 Day Chick.

Anti-brain sera absorbed with extracts of	Test antigens (saline extracts)						
	Blood	Brain	Liver	Heart	Muscle	Yolk	Saline (Control)
Unabsorbed	+*	+	+	+	+	+	—
Blood	—	+	+	+	+	—	—
Brain	—	—	—	—	—	—	—
Liver	—	±†	—	±†	—	—	—
Heart	—	—	—	—	—	—	—
Muscle	—	±†	—	—	—	—	—
Unabsorbed	+	+	+	+	+	+	—
Normal serum§	—	—	—	—	—	—	—

* In 4 of 11 antisera the reactions with blood and yolk extracts were very weak.
† ± indicates that both negative and positive results were obtained. Explanation in text.
‡ Saline added in proportion to the greatest volume antigen used for absorption.
§ From uninjected rabbits.

for one hour during which the temperature never rose above 6°C. The slightly milky supernatant (brain extract) was stored at 3° to 5°C.

Twelve rabbits were injected intravenously with 0.5, 1.0, 2.0, 4.0, and 6.0 ml of the brain extract on alternate days. After an interval of 4 to 5 weeks a second series of injections was given, beginning with 0.5, 1.0, and 2.0 ml intraperitoneally, followed by the intravenous series already mentioned. Ten to 14 days after the last injection animals were bled from the heart.

Test antigens. Heart, muscle, liver, and the blood were obtained from the same chicks used to get brain, and were lyophilized and stored in the same manner as brain. The heart, and muscle from the upper leg, were cut out with scissors and washed briefly in saline before freezing. The liver was perfused while the lobes were gently moved about, until it was a butter-yellow color. The blood was obtained by cutting a window into the air space, pipetting 1 ml of 2.5% sodium citrate over the membrane covering the chorioallantois, and then puncturing several large blood vessels in the latter. Yolk was taken by pipette from the center of yolk of non-fertile eggs. The lyophilized test antigens were extracted in the manner described for brain.

Chick embryos from 3 developmental stages (primitive streak, early neural plate, and 4 to 5 somites) were used as test-antigens. The embryos were washed in 3 changes of saline, spread on glass slides, and the stage of development checked with a dissecting microscope. Excess fluid was drained off with a blotter, and the embryos were frozen and lyophilized. The dried tissue was scraped from the slides with steel needle-knives and extracted with saline added in the same proportions as for other test-antigens.

Precipitin tests. The test antigen extracts were too cloudy to be used in ring (interfacial) tests. Each antigen was therefore diluted to the point where a precipitation ring was readily seen. This dilution was considered the stock extract and from it dilutions of 1 to 8, 48, 144, 288 and 432 were prepared with saline. Approximately 0.08 ml of the test-antigen dilutions was layered over an equal volume of antiserum or normal serum in tubes of 3.5 mm inside diameter and 3 cm tall. The tubes were incubated at 37°C for ½ hour, stored at 18°C for 30 to 40 minutes, and read with fluorescent lighting against a black, opaque background.

Absorption of Antisera. Since the anti-brain sera cross-reacted with all the test-antigens, absorptions were carried out with each test-antigen separately, using the stock extracts. 0.5 ml of antiserum was mixed with 0.2 ml of each stock extract, and allowed to stand at room temperature for 15 minutes with frequent agitation. The precipitate was centrifuged down and the clear supernatant tested against the dilutions of the test-

TABLE II.
Ring (Precipitin) Tests with Anti-brain Sera Layered, Over Extracts of Early Embryos (Chick).

Anti-brain sera absorbed with extracts of	Test antigen (saline extracts)							
	Primitive streak	Early neurula	4 to 5 somites	Brain	Blood	Yolk	Egg-white	Saline (control)
Unabsorbed*	+	+	+	+	+	+	+	—
Blood	+	+	+	+	—	—	—	—
Brain	—	—	—	—	—	—	—	—
Liver	—	—	—	—	—	—	—	—
Heart	—	—	—	—	—	—	—	—
Normal serum†	—	—	—	—	—	—	—	—

* Unabsorbed antiserum diluted with saline in proportion to greatest volume of antigen used for absorption.
† From uninjected rabbits.

antigens. Absorption was repeated until ring tests were negative for all dilutions of the test-antigen used for absorption. The absorbed antisera were then tested against the test antigens. A plus sign in Tables I and II indicates a positive test in one or more dilutions of test-antigens.

Results. Table I shows that blood extract removes precipitins for yolk and egg-white as well as for blood. However, such blood-absorbed antisera retain precipitins for organ extracts. These results were obtained with all 11 antisera tested (one rabbit died during the injection series). This indicates that the brain contains one or more constituents antigenically distinct from those of blood, yolk, and egg-white. The antigen or hapten involved is not, however, organ-specific in the sense of being limited to brain alone. It should rather be referred to as a general organ antigen, since a majority of the anti-brain sera lost activity for all test-antigens after absorption with heterologous organs. Of the 6 antisera absorbed with brain, heart and muscle, 4 showed a complete loss of precipitins for all the test-antigens. In the 2 remaining antisera, one showed weak activity for heart and brain extracts after absorption with liver, while the other showed clear activity for brain after absorption with muscle extract.

The results given in Table II, obtained with 3 antisera, show that the early embryo contains one or more constituents antigenically distinct from those of blood and yolk. Since absorption of the anti-brain sera with brain, heart, or liver, removes precipitins for the embryonic tissue as well as for these organs, it must be concluded that the embryo contains the same general organ antigen (or combining groups) present in the differentiated organs. The early stages of the organism thus contain an antigen(s) similar to, or identical with, the vitelloid constituents of adult serum,[1] and another common to several and perhaps all differentiated organs.

Discussion. Whatever may be the specific source of the organ antigen, it seems, like the blood antigens, to be soluble in saline. This could not be deduced from the injection antigen which was a slightly turbid suspension. However, clear extracts were prepared from both adult organs and the early embryos by carefully pouring saline over the tissue so as to avoid dispersion of fine particles, and extracting in the cold without agitation for 48 to 72 hours. The clear supernatants gave positive ring tests with anti-brain serum absorbed with blood extract. Also in favor of the saline-solubility of the organ antigen is the fact that brain residue remaining after thorough extraction with saline, does not remove precipitins for any of the antigens tested. The specificity of the organ antigen as distinct from blood and yolk antigens, may be due to a lipoid (hapten) constituent since Heimann and Steinfeld[2] found that alcoholic extracts of brain can be used to produce antisera which are, in some instances, specific for both alcoholic

2 Heimann, F., and Steinfeld, J., *Z. f. Imm. forsch.*, 1928, **58**, 181.

and aqueous extracts of brain although cross-reactions with liver and heart occur with some antisera. Witebsky and Steinfeld[3] and Kopeloff and Kopeloff[4] found that anti-brain sera react with alcoholic extracts of brain.

Witebsky and Szepsenewol[5] found the Forssmann antigen in chicks at 24 hours incubation. Krichevsky[6] could not detect the antigen before the fourth day of incubation but he used aqueous suspensions whereas the above authors used alcoholic extracts. By the sixth incubation day, the Forssmann antigen can be detected in desanguinated chicks but not in their blood.[7] The antigen occurs in both blood and tissues of the adult.[8] The organ antigen involved in the present study is probably not the Forssmann antigen since the activity of the antisera for tissue extracts was not removed by thorough absorption with adult blood, which contains the Forssmann antigen.

Burke *et al.*[9] presented evidence for two brain antigens, one occurring in embryos from 160 hours to at least 312 hours but not in adult brain, and a second from 260 hours incubation to the adult. Unless we may assume that the antisera used were perhaps too weak to detect the antigen in the earlier stages, it must be concluded that these authors were working with substances different from the ones involved in the present study.

Summary. Substances of common antigenic character occurring in brain, heart, liver, and muscle can also be demonstrated in the primitive streak and neurula stages of the chick embryo. These substances are distinguishable from the antigens of blood and yolk by absorption and precipitin tests and are apparently soluble in saline.

3 Witebsky, E., and Steinfeld, J., *Zentralbl. f. Bakteriol.*, 1927, **104**, 144.

4 Kopeloff, L. M., and Kopeloff, N., *J. Immun.*, 1947, **57**, 229.

5 Witebsky, E., and Szepsenwol, J., *C.R. Soc. Biol.*, 1934, **115**, 921.

6 Krichevsky, I. L., *J. Infect. Dis.*, 1923, **32**, 192.

7 Szepsenwol, J., and Witebsky, E., *C.R. Soc. Biol.*, 1934, **115**, 1019.

8 Boyd, W. C., *Fundamentals of Immunology*, 1945, Interscience Publishers, Inc.,

9 Burke, V., Sullivan, H. P., Petersen, H., and Weed, R., *J. Infect. Dis.*, 1944, **74**, 225.

16452

Infectivity of Murine SK Strain of Poliomyelitis Virus.*

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The history and characteristics of the murine SK strain have been described in several papers by Jungeblut and his associates.[1-5] One outstanding characteristic of the strain is its unusually high infectivity for mice, brain-cord virus suspensions rarely showing an ID_{50} titer under 10^{-7} by the intracranial route. This is in sharp contrast with the titers shown by the Lansing and other mouse adapted strains, but which, unlike the murine SK strain, still are paralytic for monkeys. In addition to its high infectivity by the intracranial route, Jungeblut found it infective

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1 Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407.

2 Jungeblut, C. W., and Sanders, M., *J.A.M.A.*, 1941, **116**, 2136.

3 Jungeblut, C. W., Sanders, M., and Feiner, Rose R., *J. Exp. Med.*, 1942, **75**, 611.

4 Sanders, M., and Jungeblut, C. W., *J. Exp. Med.*, 1942, **75**, 631.

5 Jungeblut, C. W., Feiner, Rose R., and Sanders, M., *J. Exp. Med.*, 1942, **76**, 31.