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Protection of Chick Against Rous Sarcoma Virus by Serum from Adult Chickens.*

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Paralleling the growth and differentiation of fowls, antibody-like factors against many antigens appear in succession in their blood with remarkable regularity.¹ The blood of new hatched chicks is entirely devoid of such factors whereas the blood of practically all adult chickens contains them. Viruses inducing tumors in fowls, such as the Rous and Fuginami sarcomas and Mill Hill 2 endothelioma, are likewise strongly suppressed by the sera of most adult chickens, but the Rous virus—the only one studied in this respect—is only occasionally suppressed by the blood serum of chicks.² Newly hatched chicks injected intravenously with the Rous and Fuginami viruses constantly developed a fatal disease characterized by hemorrhagic lesions (blebs and diffuse extravasations) in the absence of gross neoplastic lesions; pullets developed a disease of a combined sort where hemorrhagic and neoplastic lesions were observed side by side; adult chickens developed a disease less frequently, and when present, consisted in most cases of lesions of the usual neoplastic type.³ The latter results confirmed observations of previous workers on the subject.⁴ No final conclusions were reached as to the action of the sarcoma viruses on the vascular system of the chick. The fact that histological study of the earliest hemorrhagic lesions often failed to disclose the presence of tumor cells was suggestive of an effect of the virus other than neoplastic, but the conservative explanation that hemorrhages were, after all, the result of minute mural or intravascular growth could not be discarded on the evidence available.

When the Rous virus was injected into tissues of chickens of different ages the incidence and rate of growth of tumors was higher in chicks than in pullets, and higher in pullets than in adult chickens.^{2, 5} In the 2 former hosts lesions in the inoculated tissues were

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¹ Duran-Reynals, F., *Yale J. Med. and Biol.*, 1940, **12**, 361.

² Duran-Reynals, F., *Yale J. Med. and Biol.*, 1940, **13**, 61.

³ Duran-Reynals, F., *Yale J. Med. and Biol.*, 1940, **13**, 77.

⁴ Rous, P., Murphy, J. B., and Tyttler, W. H., *J. Am. Med. Assn.*, 1912, **58**, 1751; Doerr, R., Bleyer, L., and Schmidt, *Z. f. Krebsforsch.*, 1932, **36**, 256.

⁵ Rous, P., and Murphy, J. B., *J. Exp. Med.*, 1914, **20**, 419.

often hemorrhagic, and practically all of them developed metastatic lesions both neoplastic and hemorrhagic. Some of the latter were presumably induced by virus liberated from the primary lesion. Summarizing: In chicks and pullets, hosts with blood devoid of neutralizing power for the Rous virus, certain cells of the vascular system are attacked by the virus with the production of hemorrhagic lesions, and tumors grow very fast in their tissues. On the contrary in adult chickens, hosts with blood possessing a strong neutralizing power for the Rous virus, the cells of the vascular system are either insusceptible or protected against the virus, and tumors grow comparatively slowly in their tissues. Moreover,² in the latter hosts, the pronounced individual differences in the size of tumors following inoculations of virus in their tissues were shown to be in an inverse relation with the neutralizing power of the blood for the virus.

Experimental: Chicken serum was procured from normal Plymouth Rocks 8 months old, the serum from 10 chickens being pooled in aliquots. As a source of Rous virus fresh tumor tissue was extracted with 19 parts of saline solution, and the extract passed through a Berkefeld N candle. In general the experimental procedure was as follows: new hatched Plymouth Rock chicks were first injected in the peritoneal cavity with 0.5 cc of chicken serum and, as a control, as many chicks were similarly injected with either saline solution or rabbit serum. Next day the chicks were injected in the jugular vein with 0.5 cc of tumor filtrate, and the peritoneal injections of sera or saline were repeated daily until the first chick died, usually from 8 to 12 days after the injection; then the rest of the animals were sacrificed and studied. If none of the chicks had died before 12 days they were likewise sacrificed. Departures from this technique will be described as the occasion arises.

Out of 12 experiments thus carried out protection against the effects of the virus by treatment with chicken serum were obtained in 9. These comprise a total of 78 treated chicks and 80 controls. In Table I results are grouped according to: (a) Incidence and type of

TABLE I.
Protection of Chicks Against the Effects of the Rous Virus by Treatment with Serum from Adult Chickens.

Treatment	Total	Lesions around injected vein	Generalized lesions			Blood clot in peri- toneum	Death within 12 days
			Hemor- rhagic	Com- bined	Neo- plastic		
Chicken serum	78	18*	19	11	4	9	0
Rabbit serum or saline sol.	80	41†	43	18	1	26	17

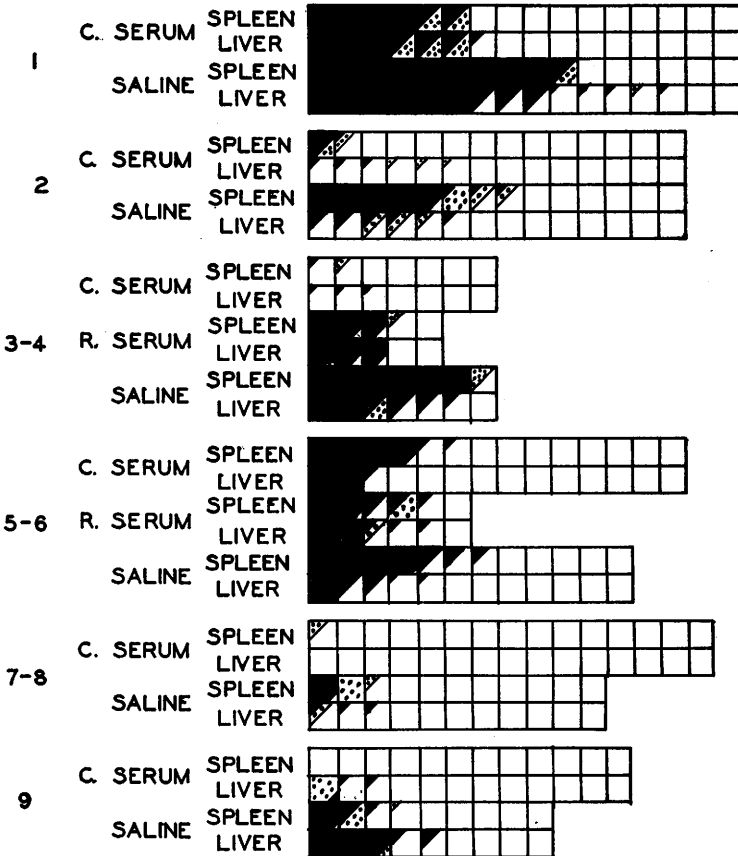
*All neoplastic.

†6 hemorrhagic, 35 neoplastic.

gross local lesions, (b) incidence and type of gross generalized lesions, and (c) incidence of death within 12 days following the virus injection. These results will be analysed separately.

(a) *Local Lesions.* The lesions which develop around the injected jugular vein are the result of soiling the tissues by minute amounts of virus. As described elsewhere³ they are of 3 sorts: purely hemorrhagic (blebs); combined hemorrhagic—neoplastic; and purely neoplastic. Besides the differences indicated in Table I, the tumors in the treated group were smaller than those of the control group; in either group they had at best an area of 1 sq cm. In a more direct study of the influence of the treatment upon occurrence of local tumors, the following experiments were carried out.

EXP NO.



TEXT-FIGURE 1.

Each square represents one chick; a wholly black or dotted square indicates an organ studded with hemorrhagic and neoplastic lesions respectively while lesser degrees of hemorrhagic or neoplastic involvement are indicated by correspondingly smaller black or dotted fractions of the square. C—chicken; R—rabbit.

Experiments. In 3 different investigations 52 chicks in 2 groups were injected intradermally in the breast with 0.5 cc of a 1:200 dilution of filtrate of Rous tumor. Half the animals were treated daily with chicken serum and the other half with saline solution, as above described, for 20 days. Tumors resulted in most cases, and they were of the same size in both groups. The study was repeated on 14 chicks with the only difference that they were given 8 daily peritoneal in-

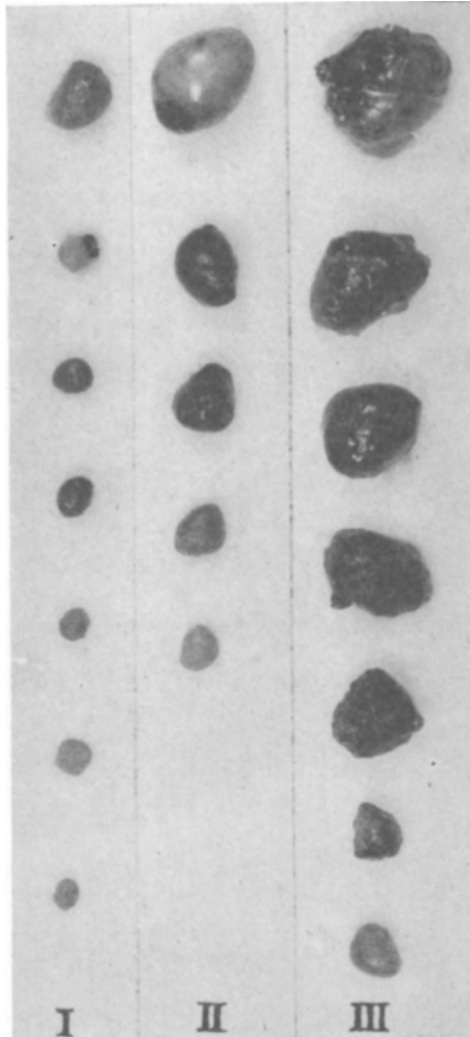


PLATE 1.

Experiments Nos. 3 and 4 in Text-fig. 1. Spleens from chicks treated with (I) normal chicken serum, (II) normal rabbit serum, and (III) saline solution.

jections of 0.5 cc of chicken serum before and 25 after the virus was injected intradermally. Results were as follows: 50% of the chicks in the treated group and 88% in the control group died of their tumors, the average areas of the tumors being 5.5 and 12 sq cm respectively. The effect of the treatment on the incidence and type of metastatic lesions will be taken up in the following section.

(b) *Generalized lesions.* These had been fully described elsewhere.³ Since the organs constantly affected are the spleen and liver, the differences in their involvement, expressed graphically in text-figure I, and Plate I can be taken as a faithful index of the intensity of the infection.

Moreover, 12, 10 and 3 chicks in the treated group against 21, 25 and 16 in the control group showed lesions in the lungs, gonads and intestines respectively; in gonads and intestines they were hemorrhagic (blebs) in both groups; in the lung (diffuse extravasations) were always hemorrhagic in the control group, but sometimes neoplastic in the treated group. Study of the chicks that died of their skin-induced tumors, in the foregoing section, showed that treatment with chicken serum also afforded a protection against or changed the type of the metastatic lesions. In the treated group the lungs were replaced by tumor, the livers showed equal proportions of tumors and blebs, but the spleens were free of disease. In the control group the lungs—always neoplastic—showed also extensive extravasations of blood, and the livers, and also the spleens, showed exclusively hemorrhagic lesions.

In the 3 experiments where no protection against the virus injected intravenously was observed the tumor preparations employed were unusually active. In another experiment a single dose of serum (5 cc) was injected intraperitoneally shortly after a virus preparation of medium activity was injected in the vein. No protection was obtained.†

(c) *Time of death.* Longer survival in the treated group is clearly suggested, in Table I, by the fact that all the 17 chicks that died belonged to the control group. The fact was directly shown by an experiment (9 in text-fig. I) where chicks were given 8 daily peritoneal injections of 0.5 cc of chicken serum preceding, and 20 more

† This particular experiment can, in a way, be compared to that carried out by Rous and Murphy⁵ in which from 35 to 65 cc of blood from immune fowls were transfused in one time to 5 fowls bearing Rous tumors, and the growths were not affected. Both experiments pose the questions whether the disease in the adult could be controlled by daily injections of serum as the disease in the chick is; presumably the experiment, if attempted, would require almost prohibitive amounts of serum.

following the intravenous injection of 0.5 cc of tumor filtrate at 1:200. The control chicks were similarly injected with saline solution. In the treated group 3 of the 12 chicks died 25, 36 and 38 days after the virus injection, and when the remaining chicks were killed 50 days after this injection, no lesions were found in them. In the control group 5 out of the 9 chicks died 10, 15, 16, and 20 days after injection of virus, and in 2 of the remaining 4, killed after 50 days, actively growing tumors were found.

Discussion and Summary. Whatever vascular cells are affected, the induction of hemorrhagic lesions by the Rous virus indicates extreme susceptibility such as that usually found in the chick.³ The present results show that adequate treatment of such hosts by serum from adult chickens—presumably through the antibody-like factor there present—causes either a shifting from hemorrhagic into neoplastic lesions or suppression of all lesions. In other words the treated chicks react to the virus much as adult chickens do. This goes to show that in fowls the age differences in the response to the Rous virus are dependent upon factors in the blood, and confirms the supposition that the relative ineffectiveness of the virus upon the vascular system of the adult is due to the selective protection by the blood of the susceptible cells of the vascular wall whereas the susceptible extravascular cells are less protected. Presumably these cells in the chick are still less protected since after an injection of virus, large tumors always arise from them.² The present results show that treatment with adult's serum is also effective in protecting these extravascular cells as long as the virus has been injected in the tissues in very small amounts, as presumably happens in the procedure of the intravenous injection. or when the chicks have been prepared for several days before larger amounts of virus were injected intradermally.