

were 4 to 5 months old and were inoculated intravenously with 0.6 cc. of leucemic blood. Of 6 Barred Rocks, 4 acquired leucosis; of 6 White Leghorns, 4; of 6 Rhode Island Reds, 2; of 6 mixed breed, 2. In another series of inoculations of 6 Bantams, all acquired leucosis.

Of the gallinaceous birds, other than domestic fowls, the Guinea fowl was tested and all of the 10 young Guinea fowls that were injected resisted the inoculation. Likewise unsuccessful were the attempts to transmit leucosis to 12 pigeons.

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Fate of Leucemic Blood of Fowls After Transfusion.*

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Leucemic blood was transferred into healthy animals and the fate of the immature leucocytes was followed in the circulating blood and in the blood forming organs of the recipient. Such a study might reveal whether leucemia is the result of multiplication of the transfused immature leucocytes in a susceptible host. In addition, the fate of the transmissible agent in the blood of the recipient was studied because it was assumed that if leucosis were secondary to infection, the infective agent might be recovered from fowls that exhibited no leucemic blood changes.

The fate of transfused leucemic cells. Six young chickens were transfused with 20 to 35 cc. of the blood of fowls with severe myeloid leucemia, after 13 to 35 cc. of blood had been removed from the circulation of each recipient. Following transfusion, there was an immediate increase of the leucocyte count. Leucocyte counts of the blood of donors and recipients, mixed *in vitro* in proportions estimated to be those in the recipients, gave values from 2 to 3 times as high as those found in the recipients 30 minutes and 1 hour after transfusion. Figures for red cells and hemoglobin in the mixtures, on the other hand, agreed roughly with the figures found in the transfused animal. This observation indicated that leucocytes had been removed from the peripheral circulation. There was a slight further fall in the leucocyte counts during periods

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varying from 1 to 2 days, which was followed by a slight rise during a period of about one day. Subsequently the leucocyte count of 4 of the 6 transfused chickens fell to normal within a few days with a complete disappearance of the immature cells. Three of these 4 birds showed no further changes, but one developed erythro-leucosis 47 days after the transfusion.

In 2 of the transfused chickens there was, instead of a drop, a rapid rise in the number of the circulating leucocytes, and both birds died in 3 days with the blood picture of leucemia. In one, the leucocyte count before transfusion was 27,500, one half hour after transfusion, 365,000, and in 3 days, 910,000.

This secondary elevation was much higher than could be attributed to the mechanical effect of transfusion. The large number of mitoses in the circulating blood gives further evidence that active multiplication of the transfused leucemic blood cells had occurred in the body of the host. This opinion was strengthened by microscopic examination of the organs. There was engorgement of the blood vessels with primitive large mononuclear leucocytes, and many of these cells were undergoing mitotic division. The bone marrow contained abundant fat and showed only a slight degree of hyperplasia. There were small foci in the liver resembling myeloid metaplasia. The changes observed are best explained by assuming that the host acted as an incubator for the leucemic blood introduced into its circulation.

Fate of the transmissible agent. Five young chickens were injected intravenously each with 0.5 cc. of the blood of a fowl with severe myeloid leucemia. Blood was removed from the veins of 3 of these chickens 30 minutes, 1, 7, and 15 days after inoculation and injected immediately into the veins of normal chickens in amounts of 0.5 cc. Fifteen days after inoculation, only one of them remained normal. Each of 4 normal chickens were injected with 0.5 cc. of blood, from this apparently healthy fowl. Each of 5 fowls were inoculated with 0.7 cc. blood of an apparently healthy fowl transfused 30 days previously with 20 cc. leucemic blood.

These experiments have shown that the transmissible agent is demonstrable in the blood stream of the recipient 30 minutes after inoculation. Most, if not all of it, leaves the circulation within 24 hours and reappears within 7 to 15 days in fowls that develop leucosis. In fowls that remained free from leucosis, the transmissible agent was not found in the circulating blood.

Previous observations¹ gave evidence that avian leucosis can be

¹ Furth, J., PROC. SOC. EXP. BIOL. AND MED., 1931, 28, 449.

produced by cell-free material. The present study indicates that the leucemic cell itself is capable of autonomous growth.

Rous² observed that tumor cells and their filtrates behave differently when brought in contact with immune sera, the former being neutralizable, the latter not. Similar observations were made by Sittenfield, Johnson and Jobling.³ It may be assumed that this difference is due to the position of the agent, intracellular in one instance or free in the other. Our observations suggest the occurrence of 2 distinct processes: (a) a filterable agent causes neoplastic growth and (b) its product, the neoplastic cells are capable of autonomous growth.

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Effect of Suprarenal Cortical Extract on Nitrogen and Sugar Elimination in Depancreatized Dogs.

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In previous publications we¹ described a new calorigenic principle obtained from the suprarenal cortex or whole gland. The active substance is combined in the lipoid fraction and is extracted from the tissue with ethyl alcohol. After evaporation of the alcohol and extraction of the residue with benzene or ether, the active lipoid protein-free fraction is obtained. The water-soluble fraction of the lipoid contains epinephrin besides the active substance.² Epinephrin, however, is less firmly bound by the lipoid and can first be removed by prolonged washing or mild acid hydrolysis in the absence of oxygen. The active principle is unstable, but in the absence of oxygen we have kept a preparation active for 5½ months. In our earlier work the extract was administered orally but effects were not always constant. Given hypodermically, approximately one-tenth to one-fifteenth the amount is required and the results have been much more consistent.

² Rous, P., *J. Exp. Med.*, 1913, **18**, 416.

³ Sittenfield, M. J., Johnson, B. A., and Jobling, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28**, 517.

¹ Koehler, A. E., *Proc. Soc. Exp. Biol. and Med.*, 1928, **26**, 296. Koehler, A. E., and Eichelberger, L., *Am. J. Physiol.*, 1929, **90**, 2.

² Koehler, A. E., and Eichelberger, L., *J. Biol. Chem.*, 1930, **87**, 38.