found to be within this limit. In 3 cases of nephrosis with edema a distinctly red color was obtained and the protein digestion method showed contents of 3.1, 2.9 and 3.4 gm. per 100 cc. respectively. The turbidity of the sera due to the high lipoid content did not interfere. One case of suspected nephrosis with slight edema showed an orange red color equivalent to a buffer of pH 5.2 and was shown by the standard methods to have 4.5% of protein. Certain of the sera which gave a yellow orange color (pH = 5.8) contained about 6% total protein and were to be classified as low normals. In cases of cachexia of cancer an orange pink color (pH = 5.3) was obtained and the sera showed a protein content of 4.6% and 4.8% respectively.

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Concentration of the Causative Agent in the Filtrate of the Rous Chicken Sarcoma.

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The studies of Lewis and Andervont,[1] and those of Michaelis,[2] also of Baker and MacIntosh,[3] Frankel[4] and Sugiura and Benedict,[5] within the past 2 or 3 years, have disclosed additional information concerning the physical and chemical properties of the causative agent of the Rous Chicken Sarcoma. Most of these observers investigated the range of H ion concentration in which the active agent retains its infectivity, and concluded that the nature of the buffer solution appears to be of no great significance, as long as the pH is between 3.8 and 10 to 11. Since the experiments of Lewis and Michaelis were carried out with tumor extracts, and those of Sugiura and Benedict with fragments of tumor tissue, we were interested in determining the activity of the causative agent in a cell-free filtrate under varying pH values, and to correlate this with the results obtained with the tumor extract, or tissue fragments.

[1] Lewis and Andervont, *Bull. Johns Hopkins Hosp.*, 1927, **41**, 185.

[2] Lewis and Michaelis, *Bull. Johns Hopkins Hosp.*, 1928, **43**, 92.

[3] Baker and MacIntosh, *Brit. J. Exp. Path.*, 1927, **8**, 257.

[4] Frankel, E., *Z. f. Krebsforschung*, 1929, **29**, 491.

[5] Sugiura and Benedict, *J. Can. Res.*, 1927, **11**, 164.

We found that buffering the active filtrate with a potassium hydrogen pthalate solution to pH 4 caused the solution to become cloudy, with subsequent precipitation upon standing. Upon centrifuging the suspension for about 30 minutes, a heavy precipitate was formed, and the supernatant fluid became clear. The clear supernatant fluid when tested by inoculation into chickens, is non-infective. Only when it remains slightly cloudy, indicating that all of the protein substances have not been carried down in the precipitate, will an occasional tumor result after a much delayed period of time. The precipitated portion while still at pH 4 when injected into chickens is only slightly infective, that is, its infectivity seems somewhat reduced. In other words, the active agent is carried down in the precipitated portion, and rendered partly inactive at this H ion concentration.

When the supernatant fluid is decanted, and the remaining precipitated residue is washed several times with a buffer solution at pH 4, and then resuspended in a Clarke's standard buffer solution adjusted to pH 8, the causative agent is liberated, and the supernatant fluid becomes so highly infective that doses of 0.2 cc. will cause a tumor in from 8 to 11 days. The residue from this washing at pH 8 is also slightly potent.

The potency of the causative agent in the supernatant fluid at pH 8 can again be inhibited by bringing the solution to pH 4, for at this degree of acidity, a precipitate again forms, which contains the active principle, and the supernatant fluid becomes inactive. The non-potency of the supernatant fluid at pH 4 is not due to the acidity for when neutralized, it still fails to produce a tumor.

After 3 or 4 re-precipitations of the agent at pH 4, the bulk of the final precipitate may be reduced to 1 to 2 mg., dry weight, whereas the first precipitate might weigh 30 mg. The infectivity of this concentrated precipitate after it has been extracted by a buffer solution at pH 8 equal in volume to the original filtrate, is not only fully retained, but even seems to be enhanced. For example, where it required 0.5 cc. of the original filtrate to obtain a tumor, the concentrated precipitate suspended in the same volume of fluid at pH 8, gave rise to tumors in doses of 0.2 cc. At times, the washings from the first precipitate gave tumors with 0.1 cc. The agent is evidently more virulent in the partially purified form than in the original filtrate, as it killed the chicks in about 20 days, whereas the length of life of the chicks after inoculation with the original filtrate averaged 28 days.

The moist concentrated precipitate will maintain its infectivity

when kept on ice for 4 to 5 days. Moreover, when dried in the desiccator *in vacuo,* and kept for 10 days or longer, it is still potent, though its infectivity is considerably lessened. As a parallel experiment, the filtrate itself was dried in the desiccator and found to be non-potent after 24 hours.