tion decreases, and it does not occur when the serum is diluted beyond 1:128.

2. *Agglutination of human red blood cells and bacteria.* In accordance with the work of Gutman and others we found that solutions of basic dyes agglutinate red blood cells and bacteria.

3. *Protective power of human serum.* Whole human serum added to a 2% suspension of human red blood cells or bacteria and thoroughly mixed with them acted as a protective and prevented their agglutination upon the subsequent addition of the gentian violet solution. The serum exerted this protective power in dilutions up to 1:64. In higher dilutions the serum no longer protected and agglutination occurred as if no serum were added.

4. *Desagglutination by serum.* A 2% suspension of human red blood cells was agglutinated by our solution of gentian violet. Equal amounts of whole human serum, or serum diluted up to 1:8 added to the cells agglutinated by the dye caused prompt desagglutination.

5. *Flocculation of spinal fluids.* Our solution of gentian violet added to undiluted spinal fluid or its dilutions up to 1:5 caused their flocculation. In dilutions higher than 1:5 flocculation decreases and does not occur when the spinal fluid is diluted beyond 1:10.

6. *Protective power of spinal fluid.* Undiluted spinal fluid added to a 2% suspension of human red blood cells or to bacteria protects them against agglutination upon subsequent addition of the gentian violet solution.

There are individual variations in the reactivity of human red blood cells, sera and spinal fluids towards dyes. We are now carrying on further experiments to determine whether there are any differences produced by pathological conditions in the human body.

5399

Demonstration of a Tumor-Inhibiting Substance in Filtrate of Rous Chicken Sarcoma and in Normal Chicken Sera.

MAURICE J. SITTENFIELD, BALBINA A. JOHNSON AND JAMES W. JOBLING.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

In a previous communication[1] we reported that the active agent

[1] Sittenfield, M. J., and Johnson, B. A., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 206.

in the Rous chicken sarcoma filtrate could be concentrated and partially purified by adjusting the filtrate, normally about pH 7.2, to a reaction of pH 4. When brought to this reaction with a phthalate buffer, the tumor-producing agent is carried down with the precipitated portion. When this precipitate is extracted at pH 8, the carcinogenic agent is not only set free, but the activity of the extract seems definitely greater than that of the original filtrate.

To account for the increased activity of the first extract at pH 8, several possibilities suggested themselves; among others, that the active agent in the filtrate may be in combination with substances that restrain its activity to some extent, and that in the process of precipitation by the method described above, there is brought about dissociation between the restraining or protective agent and the tumor-producing element. If true, this would explain why the extract of the first precipitate is considerably stronger than the filtrate from which it was prepared.

To test for the presence of a protective agent, we examined the supernatant fluid obtained after precipitating at pH 4 the active agent from a 10% filtrate. After centrifuging, the supernatant fluid was decanted, neutralized and then concentrated *in vacuo* at room temperature to ½ its original volume. Two cubic centimeters of this concentrated supernatant fluid were now mixed with 0.5 cc. of the original filtrate, containing approximately 2 infective doses, and allowed to stand for 30-40 minutes prior to injection. Chicks, 10-14 days old, were used in all the experiments, for they have been found to give more uniform results. Of 80 chickens inoculated with this mixture, 75% failed to develop tumors, while tumors developed in all the controls which had received this amount of filtrate alone. It was found that smaller doses of supernatant fluid were ineffective, as they gave protection only in rare instances.

In the 25% which developed tumors, the inhibitory effect of the supernatant fluid was shown by the fact that the appearance of the tumors was delayed, they grew less rapidly, did not attain their usual size, and in one or 2 instances receded. When 8 or 10 times the required dose of the active agent is used, with a proportionate increase in the amount of supernatant fluid, no inhibitory effect is observed. With a very active extract of a pH 4 precipitate, increased amounts of the concentrated supernatant fluid must be used, since we found that 2 cc. will not neutralize more than approximately 2 infective doses.

In other words, the first precipitation had brought about a separation between the active agent now held in the precipitate and a pro-

tective fraction which remains behind in the first supernatant fluid. It seemed probable that this protective substance would be present only in the supernatant fluid from the first precipitation at pH 4, and this was found to be true, for the supernatant fluids from the second and subsequent precipitations proved to be non-inhibitory.

By half saturating the supernatant fluid with ammonium sulphate and then dialyzing against running water, it was found that the protective substance was confined to the globulin fraction. In no instance was an inhibiting action obtained with the albumen fraction.

In view of the observations of Rous[2] and Fischer[3] that antisera will protect against inoculations with the free virus but not against the tumor cells, experiments were made to determine if this inhibiting action of the supernatant fluid would be effective in preventing the growth of tumor fragments. Experiments were conducted similar to those described with the filtrate but in no instance were we able to find that the inhibiting substance was effective in preventing the growth of the living tumor cells. These observations are in line with the results obtained by others who have worked with filterable viruses, who claim that immune bodies can be obtained that are effective against the virus outside the cells, but ineffective once the cells are infected.

These observations on the protective substances in the tumor filtrate suggest the probable presence of similar substances in the blood, as claimed by Fischer[3] who, however, does not describe very clearly his methods of demonstrating the presence of the inhibiting action of normal sera. Andrewes[4] and Mottram,[5] using small doses of serum, were unable to demonstrate neutralizing substances in normal chicken sera, but did find them in chickens bearing slow growing tumors. We tested the protective action of the sera of normal and tumor-bearing chickens against the active agent in the Rous chicken filtrate, and found that the serum of normal chickens was protective if used in amounts of approximately 2 cc. to 4 cc. against one or two infective doses. In testing the serum of a chicken partially protected by the injection of a mixture of 2 cc. of concentrated supernatant fluid and 0.5 cc. filtrate, in which the tumor appeared after 19 days and grew very slowly, we found its inhibitory effect was distinctly greater than that of sera of normal chickens.

---

[2] Rous, Peyton, *J. Exp. Med.*, 1913, **18**, 416.

[3] Fischer, Albert, *Zeits. m. Krebsforschung*, 1926, **24**, 580.

[4] Andrewes, C. H., *J. Path. and Bact.*, 1931, **34**, 91.

[5] Mottram, J. C., *Brit. J. Exp. Path.*, 1929, **9**, 147.

This chicken was bled 36 days after the inoculation. There are indications that the sera of tumor-bearing fowls in the terminal stages of the disease are not protective to the same degree, but this point is now under investigation. Here, too, as in the chicken filtrate, the protective substance seems to be associated with the globulin fraction. The failure of Andrewes and Mottram to find neutralizing substances in the sera of normal chickens is probably due to the small doses used. In our experience, rarely was a serum active in amounts of less than 2 cc. to 4 cc.

It will probably be found that this protective mechanism is not specific in its action, and that it is equally effective against the filtrates of other filterable chicken tumors. The development of metastases probably depends, as is customarily believed, upon the migration of live cells, for the active agent liberated by the breaking down of tumor cells is probably neutralized by the protective substances in the plasma.

5400

The Gastric Hunger Mechanism. II. The Effect of Diet.

MICHAEL G. MULINOS.

From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University.

Balloon tracings of the insulin gastric hypermotility cannot be distinguished by inspection either from the type III hunger contractions, as classified by Carlson,[1] or from the post-feeding tracings described by Mulinos.[2] They can be readily identified, as follows: The post-feeding tracings are uninfluenced by food, or by the intravenous injections of glucose; the hunger type III is depressed by food, but not by glucose intravenously; the post-insulin hypoglucemic hyperactivity is abolished by raising the blood sugar, and removing the hypoglucemia. They are all abolished by adequate doses of atropine.[2]

Two series of 4 female dogs, with gastric fistulae, were studied for 6 to 18 months. The dogs were trained to lie quietly, while the gastric contractions were being recorded by the balloon method described elsewhere,[3] using a chloroform manometer. The insulin

[1] Carlson, A. J., *Am. J. Physiol.*, 1912, **31**, 151.

[2] Mulinos, M. G., *Am. J. Physiol.*, 1927, **83**, 115.

[3] Mulinos, M. G., *Am. J. Physiol.*, 1926, **77**, 158.