Some of the experiments reported above were also performed using bacterial antigens. The results were similar to those obtained when foreign red cells were used.

Finally, attempts were made to repeat the experiments of Meyer and Loewenthal,[6] who reported antibody production in hanging drop cultures of spleen taken from rabbits injected one hour previously with bacterial antigens. Although all the older procedures were carefully adhered to, the results were entirely negative.

In view of the positive results obtained when the antigen was allowed to remain for two to three days in the animal, the negative results obtained when this period was shortened have a very definite significance. They not only suggest that antibody production is a more complicated process than is usually assumed, but they imply that it is not easy to demonstrate a production of antibodies *in vitro* unless the tissues have first been acted upon by some unknown mechanism within the body.

All the serological tests in the course of these experiments were made under the supervision of Dr. Karl Landsteiner, upon whose experience the plan of investigation was entirely dependent.

RAYMOND C. PARKER

ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK, N. Y.

PREPARATION OF AN ACTIVE AGENT FROM INACTIVE TUMOR EXTRACTS

THE majority of fowl tumors of spontaneous origin which have been transplanted have proved to be transmissible by cell-free filtrates or desiccates of the tumors. On the other hand, chemically induced fowl tumors, with the exception of three reported by MacIntosh,[1] are transferable only by grafts of living tumor cells. In this respect the latter resemble the mammalian tumors. It has been shown in this laboratory that not infrequently the low activity of filtrates or desiccates, occasionally encountered in transmitting the filterable tumors, is due to the presence of an inhibiting factor rather than the absence of the agent. This was established by the fact that the removal of the inhibitor by adsorption on alumina gel rendered the extract highly active in tumor production.[2] However, this method failed to explain the non-filtration of a slow-growing fibro-sarcoma (Chicken Tumor 10) which has been under observation in this laboratory for the past ten years. A strong inhibitor has been shown to be present in this tumor by cross tests with Chicken Tumor I, but treatment of extracts with alumina gel failed to render the extracts active in the transmission of the tumor. Yet there was a suggestion that the tumor possessed a transmissible agent from the fact that once or twice tumors resulted from injection of desiccates, but this was a rare occurrence and there was no clear-cut transmission by filtrate in the many attempts which have been made.

In recent studies of Chicken Tumor I, it has been shown that the agent could be sedimented by high-speed centrifugation, and this method seemed clearly to separate the agent from its own inhibitor.[3] This observation suggested a further attempt to transmit the fibro-sarcoma.

Experiment: A water extract was prepared from an eight-week old Chicken Tumor 10 and the extract passed through a Berkefeld V candle. The filtrate was submitted to a centrifugal force of 14,000 times that of gravity for 2½ hours. The resultant sediment was taken up in Tyrode's solution and redeposited at high speed. This washed sediment was next suspended in a volume of Tyrode's equal to 1/10 that of the original filtrate. For activity tests, 0.4 cc of the suspension were injected into normal hens, the same birds also receiving the same amount of the original filtrate in another area for control. No tumors developed as the result of injection of the unspun filtrate. Tumors did arise in 50 per cent. of the areas injected with the washed sediment, reaching a size of 3.1 × 2.3 cm within 40 days. Later some of them retrogressed. The histology of the original tumor was duplicated in the induced tumors.

In a second experiment an extract was prepared from a desiccate of Chicken Tumor 10, and this extract was treated in the same manner as that described above. Again the unspun extract gave negative results, while the injection of the washed sediment resulted in 33 per cent. positive results.

That the production of tumors in these experiments was not simply an effect of concentration of the agent was shown by the following test. The supernatant fluid, depleted of the agent, was saved. Part of the sediment, after being washed and resuspended in Tyrode's solution, was mixed with an equal volume of the original supernatant fluid. The remaining sediment was diluted to the same degree with water as a control. The injection of the diluted sediment gave positive results in 50 per cent. of the areas injected, while the part diluted with the original supernatant fluid failed to induce tumors in any of the areas injected.

The complete neutralization of the active sediment by its own supernatant fluid indicates that the failure

[1] J. McIntosh, *Brit. Jour. Exp. Med.*, 14: 422, 1933.

[2] Jas. B. Murphy, O. M. Helmer, A. Claude and E. Sturm, SCIENCE, 73: 266, 1931.

[3] A. Claude, paper in press.

of the original extracts to produce tumors was due to the presence of inhibitory elements in the solution. The fact that agent and inhibitor can be separated by physical means would suggest either that the agent is not modified by its inhibitor or that the reaction between them is easily reversible. Whether or not this centrifugation method will separate potent agents from hitherto non-filterable tumors will be determined by further tests. So far, preliminary experiments with mammalian tumors have given negative results.

ALBERT CLAUDE

ROCKEFELLER INSTITUTE
NEW YORK

THE FEEDING OF HOLLOW-SPEAR NEMATODES ON OTHER NEMATODES[1]

THE habit of preying upon nematodes has been found by the writers to be well developed in two groups of nematodes in which it appears not to have been recorded: the genus *Aphelenchoides* Fischer, 1894, and the dorylaim genera *Dorylaimus* Dujardin, 1845, *Discolaimus* Cobb, 1913, and *Actinolaimus* Cobb, 1913. Equipped with hollow, protrustive oral spears or stylets, predators of these genera feed, not as do the types formerly recognized as feeding on nematodes, but rather by inserting their spears into their prey and holding them there while sucking out the body contents. The relatively large dorylaims, with their coarse spears and powerfully muscular esophaguses, disorganize their prey so quickly that there is little opportunity for struggle. The rather small Aphelenchoides, on the contrary, feed slowly, and here the evidence is definite for one species that the prey is paralyzed almost instantly when the very slender stylet is inserted, so that struggles are prevented. During the feeding of two species of Aphelenchoides, saliva has been seen flowing out from the large dorsal esophageal gland, through the esophageal tube and into the prey.

Soil-inhabiting nematodes formerly recognized to be predacious on other nematodes, such as species of *Mononchus* Bastian, 1865, and *Diplogaster vorax* Goodey, 1929, are equipped with fine grasping teeth, with cutting teeth or with both. Slender mural teeth which slash into prey but are withdrawn while feeding are possessed by the genera *Nygolaimus* Cobb, 1913, and *Sectonema* Thorne, 1930, which are known from the work of Thorne[2] to prey upon oligochaet worms. Cobb,[3] in listing 16 genera of predacious free-living nematodes, most of which are marine forms, mentioned "Pharynx with acute clutching organs—onchia or denticles" as characteristic of such genera. Of these genera, only *Dorylaimus* feeds through a hollow oral spear.

Dorylaimus was included in Cobb's list of carnivorous forms, despite its lack of clutching organs, on the strength of his simultaneously published record of two nemas of this genus feeding upon mite eggs; of Steiner's[4] earlier record of a seta of an oligochaet worm in the intestine of *Dorylaimus regius* de Man, 1884; and of Thorne's[5] finding three instances of *Heterodera schachtii* Schmidt eggs impaled on the spears of young *Dorylaimus obtusicaudatus*.

We have repeatedly observed various species of this genus and allied genera, including larvae and adults of both sexes, feeding upon other nematodes including larvae of *Heterodera marioni* (Cornu) Goodey, 1932. All these observations, with two minor exceptions, have been in Petri dishes of agar. Unlike predators capable of grasping or slashing their prey, these forms which suck their food through hollow spears seem unable to capture prey suspended in water, for considerable pressure is required to thrust their relatively coarse, hollow spears into other nematodes. Even a very soft agar is unsatisfactory, the prey being pushed through the medium by the spear instead of being penetrated.

A predacious dorylaim finds its prey only by chance, but when its head makes contact with another nematode it responds immediately. It orients its head at right angles with the surface of the prey, so that its lips make firm contact, then protrudes its spear suddenly in an attempt to penetrate, and may do so repeatedly if not immediately successful. Once the spear enters the body of the prey it is held there while the heavily muscular esophagus of the predator begins a rhythmical sucking action which quickly disorganizes the body contents of the prey, sucking them out and leaving an empty, collapsed body wall.

A dorylaim holds its spear extended far into its prey, even during the periods of rest which alternate with periods of sucking. With a medium to large dorylaim, the spear tip reaches to the opposite body wall of such nematodes as the larvae of *H. marioni*. Such a position is advantageous in view of the long diagonally placed opening on the side of the spear.

Some of these predators have also been seen feeding on nematode eggs, both of *H. marioni* and of various free-living forms. When egg masses of the former are placed in agar dishes they prove most attractive to at least some of these species.

The predators in question have not yet been identified specifically, but it is our judgment that ten species of *Dorylaimus*, two of *Discolaimus* and one of *Ac-*

[1] Published with the approval of the director as Technical Paper No. 97 of the Pineapple Experiment Station, University of Hawaii.

[2] G. Thorne, *Jour. Agr. Research*, 41: 445–466, 1930.

[3] N. A. Cobb, *Jour. Parasitology*, 15: 284–285, 1929.

[4] G. Steiner, *Jour. Agr. Research*, 28: 1062–1064, 1924.

[5] G. Thorne, *Jour. Agr. Research*, 37: 575, 1928.