intramuscular or intravenous injections. The cause of this unusual effect of the intravenous inoculation of Rous agent in the experiments reported is entirely obscure. We do not know whether it is to be sought in the particular manner of applying the agent (repeated intravenous injections) or whether we are dealing with a real modification of the agent, which took place spontaneously or resulted from long passage *in vitro*. All these possibilities deserve consideration.

During the preparation of this note a paper of Shrigley, Greene and Duran-Reynolds[9] appeared. The authors observed that the Rous sarcoma agent after remaining in the anterior chamber of the eye of the guinea pig acquired the ability to produce periosteal tumors in chicks. To judge by the description and the photomicrograph of these growths, they are very similar to those here reported. The acquisition of a new tissue affinity was ascribed to modification of the agent caused by its sojourn in an unnatural environment.

Summary. The appearance of an osteoid sarcoma in a series of chickens injected intravenously with the causative agent of Rous sarcoma derived from sarcoma cell cultures, is reported.

[9] Shrigley, E. W., Greene, H. S. W., and Duran-Reynals, F., *Cancer Research*, 1945, **5**, 356.

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Development of Tolerance to Typhoid Bacterial Pyrogen and its Abolition by Reticulo-Endothelial Blockade.*

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Patients who are being given fever therapy by means of intravenous injections of typhoid vaccine exhibit a decreasing reactivity, and must be given larger and larger doses of the vaccine in order to develop similar fevers at successive treatments. After several injections some individuals may require doses as large as 250 ml of typhoid vaccine in a single day.[1] The mechanism of this remarkable tolerance has not been explained. The present report deals with a study of the phenomenon in rabbits.

The rabbits used were males, of mixed breed, weighing 2 to 3 kg. During test periods they were placed in wooden stalls, and held by head boards. Rectal temperatures were taken at 30-minute intervals, through openings in the floors of the stalls. Observations were never continued for more than 7 hours after giving vaccine, to prevent undue fatigue of the animals. The vaccine used contained approximately one billion killed *E. typhosa* per ml.† The dose was 1 ml of a 1:8 dilution in physiologic salt solution. The agent used for blockade of the reticulo-endothelial system was colloidal thorium dioxide (Thorotrast-Heyden Co.); 9 ml was injected intravenously.

The febrile responses to daily injections of the same dose of vaccine were recorded on 40 rabbits during periods of from 8 to 45 days. The first injection of vaccine always caused a rise in body temperature of 4-5°F, and some fever persisted throughout the 7-hour period of observation. The 2nd and 3rd injections generally caused almost as much fever as the first, but after that there was a decrease in reaction until the 6th to 10th day. Additional injections caused no further diminution, each one inducing approximately

* Aided by a grant from the Venereal Disease Division of the United States Public Health Service.

[1] Heyman, A., *Ven. Dis. Inform.*, 1945, **26**, 51.

† This vaccine was prepared in the laboratories of the Georgia State Department of Public Health. It is ordinarily used for human immunization and fever therapy.

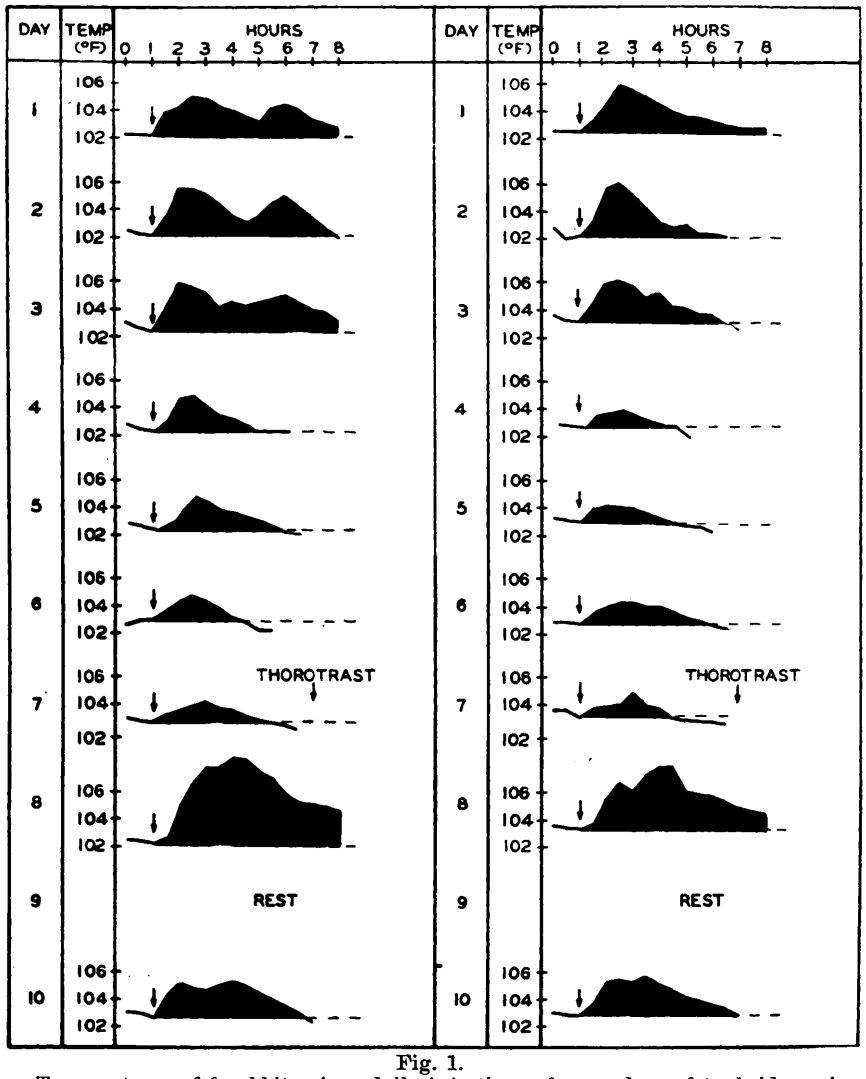

Fig. 1.

Temperatures of 2 rabbits given daily injections of same dose of typhoid vaccine. After development of tolerance, reticulo-endothelial blockade with Thorotrast caused a marked alteration in their temperature responses.

the same amount of fever, *i.e.*, a rise of 1.5-2.5°F, with return to the starting temperature in 3 to 5 hours. Four animals received daily injections for the full period of 45 days. After the usual decline in response during the first week these continued to respond to each dose of pyrogen with low grade fevers of about the same extent.

In other experiments longer intervals were allowed to elapse between injections of vaccine. Four rabbits which were injected once a week for 4 months developed some, but not a very considerable, tolerance. Another group of 4 rabbits received vaccine twice a week. A gradual decline in febrile response occurred during the first 8 to 10 injections, after which they reacted to each dose with about the same degree of fever, intermediate between an initial reaction and the minimal reaction noted in animals injected daily. At the end of 4 months the interval was shortened to one day, and they all showed a definite further diminution in their febrile responses.

Reticulo-endothelial blockade produced a

striking effect on the temperature responses of rabbits that had been previously "trained" by repeated daily injections of vaccine. A typical result is illustrated in Fig. 1. Here it will be noted that a considerable modification in response was manifest by the 7th day. Each animal was then given Thorotrast. On the following day administration of typhoid vaccine caused high, prolonged temperature elevations. After a rest of one day their febrile responses to the vaccine were again considerably lessened. This brief effect of the blocking agent is in line with other experience on functional interference with the reticulo-endothelial system.[2]

[2] Jaffe, R. H., *Physiol. Rev.*, 1931, **11**, 277.

These experiments show that rabbits can develop a tolerance to typhoid bacterial pyrogen, and that the tolerance is most marked when injections are given frequently. The mechanism of development of this state is not yet explained. Certain other observations, which cannot be given in detail here, indicate that the production of specific humoral antibodies is not responsible, and furthermore, that the development of tolerance to typhoid vaccine carries with it a similar alteration in response to other bacterial pyrogens. Possibly the process involves a change in the functional activity of the reticulo-endothelial system, providing for more rapid disposal of the foreign material.

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Effect of Penicillin on Blood Urea in the Rat

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(Introduced by Victor C. Myers.)
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Early in 1943 the Floreys[1] reported that 4 out of 5 patients treated with 120,000 or more Oxford units of penicillin manifested a rise in blood urea. The elevated values returned rapidly to normal when the drug was withdrawn. Although no explanation was advanced for this phenomenon it was not thought to be due to renal damage.

The present study demonstrates that penicillin has no significant effect on the blood urea level of normal rats even though exceedingly large amounts of the drug are administered.

The penicillin employed was a rather impure preparation assaying 300 Oxford units per mg of total solids.* Normal adult albino rats of both sexes were used and the penicillin was injected intraperitoneally in a solution containing 25,000 Oxford units per ml. Samples of blood (0.1 or 0.2 ml) for urea determinations were obtained from the tail vein directly into a pipette and the urea was analyzed by the colorimetric method described by Ormsby.[2]

The results are presented in Table I. It is immediately evident that no significant increase in blood urea results even after the administration of 500,000 Oxford units per kg of body weight. This dosage represents the largest amount of our preparation of penicillin that could be tolerated by the rat without any apparent toxic manifestations. It should be emphasized that this is a tremendously large dose. When a group of rats were given 1,000,000 Oxford units of penicillin per kg of body weight over a period of 10 hours, 4 of the 6 rats died on the same day and the 2 surviving animals were severely

[1] Florey, M. E., and Florey, H. W., *Lancet*, 1943, **1**, 387.

* Kindly supplied by Ben Venue Laboratories, Inc., Bedford, Ohio.

[2] Ormsby, A. A., *J. Biol. Chem.*, 1942, **146**, 595.