in vagina, cervix, and uterus. 2. Estrogenic hormones may cause proliferations which are not limited to one organ, but affect vagina as well as cervix and uterus. 3. In the mouse observed by us the abnormally proliferating tissue formed under the influence of these hormones was specific in accordance with the actual and potential structures of the various tissues involved.

8958 P

Precipitin Reactions of Helminth Extracts.

LESLIE L. EISENBRANDT. (Introduced by T. C. Nelson.)
From the Zoological Laboratory, Rutgers University.

The serological technic of Boyden[1] offers a suitable means of investigating the preciptin reactions of some helminth extracts, and of studying the degree of relationship indicated by the tests. The limited research in this field has been done almost entirely with saline suspensions of powdered worms. The content of these suspensions was unknown, thus prohibiting the use of definite amounts of antigen which in turn prevented comparable interpretation of the results. Schwartz[2] and Hektoen[3] studied the precipitin reactions of a few dried helminths using the best methods available at the time.

In the present work fresh worms were extracted with sterile buffered saline, and the resulting extracts were passed through Seitz filters and bottled under sterile conditions. The antisera were produced in rabbits by injecting intravenously extracts having 0.00384 gm. total nitrogen per kilo body weight; they were divided into 4 doses of increasing amounts on alternate days. None of the rabbits were reinjected. The quantitative precipitin tests, constant in titer within ± one test tube as shown by repeated tests, permit accurate readings of an antiserum with its homologous antigen and with heterologous antigens.

The nitrogen content of the helminth extracts, as determined by the Kjeldahl method, is found to be much less than that of the mammalian blood sera studied by Boyden. He finds, also, that the non-protein nitrogen content of the mammalian sera is negligible,

[1] Boyden, Alan, *Am. Nat.*, 1934, **68**, 516.
[2] Schwartz, Benjamin, *J. Parasitol.*, 1921, **7**, 144.
[3] Hektoen, Ludvig, *J. Infect. Dis.*, 1926, **39**, 342.

TABLE I.

The Titers of Precipitin Tests Expressed as Per Cent Values of the Homologous Titers with their Probable Errors below them. The Values Are Based upon Three or More Tests. Homologous titers in italics.

Antigens	Reciprocal							Non-reciprocal	
Antisera	*Toxocara canis*	*Ascaris suum*	*Ascaridia lineata*	*Dirofilaria immitis*	*Macracanthorhynchus hirudinaceus*	*Taenia pisiformis*	*Moniezia expansa*	*Moniezia alba*	*Dipylidium caninum*
Toxocara canis	*100*	12.50 ±0	0.07 ±0.006	0.001 ±0	0	0	0.02 ±0	0	0
Ascaris suum	12.50 ±0	*100*	0.10 ±0	0.05 ±0	0	.03 ±.005	0	0	—
Ascaridia lineata	0	33.33 ±3.75	*100*	0	0	0	0	0	—
Dirofilaria immitis	50.00 ±0	0	0	*100*	0	0	0	0	—
Macracanthorhynchus hirudinaceus	0	0	0	0	*100*	0	0	.31 ±.03	.46 ±.04
Taenia pisiformis	.33 ±.02	.26 ±.02	0	0	0	*100*	0	.15 ±.05	.26 ±.03
Moniezia expansa	0	0	0	0	0	0	*100*	30.03 ±0	—

whereas the opposite is found with the helminths. For example, in extracts of *Dirofilaria immitis,* the heart worm of the dog, and in *Macracanthorhynchus hirudinaceus,* the thorny-headed worm of the hog, the non-protein nitrogen content is apparently as great as the total nitrogen. For these species the total nitrogen is 0.061 and 0.072 gm. per 100 cc. of extract respectively. The biuret and glyoxylic tests for protein are negative to the *D. immitis* extract and only faintly positive to the extract of *M. hirudinaceus.* This would be expected in view of the fact that the latter contains more nitrogen which could involve a larger quantity of protein, provided the extract dilutions were at the limit of sensitivity of the chemical tests. Although the protein content of the worm extracts is low, sufficient is present to stimulate the formation of antibodies having quite high titers.

The data from a series of tests are given in Table I, which shows the degree of relationship of several parasites belonging to the phyla Platyhelminthes and Nemathelminthes. From these results it is noted that in every case the antisera react more strongly with the homologous than with the heterologous antigens. There is a parallelism between the taxonomic relationship of the helminths, as based upon morphological characters, and the precipitin tests which become weaker as the relationship grows less due to the chemical dissimilarity of the extracts. The ascarids react more strongly with each other than with the heart worm or with the cestodes. Interphylar tests occur in the case of 2 tapeworms and 2 roundworms.

There is lack of agreement between the serological and the current morphological relationship of *Macracanthorhynchus hirudinaceus,* the thorny-headed worm of the hog, which commonly is classed with the roundworms. In these tests the antiserum for the thorny-headed worm parasite does not give a single reaction with the nematodes, but gives two reactions with the cestodes. The 2 tapeworm antigens, from *Dipylidium caninum* and *Moniezia alba,* give reactions which indicate a relationship between the Acanthocephala and the Cestoda rather than with the Nematoda.

The principle of reciprocal relationship, which Boyden finds so important when using mammalian blood sera, fails to have significance in the case of the helminths. The complex chemical mixtures present in the whole worm extracts may be the cause for the lack of agreement. Blood sera on the other hand have a simpler structure which would not complicate the reactions as greatly. Agreement is a possibility in the helminths, however, as evidenced in the single case of *Toxocara canis* and *Ascaris suum* which are 12.50% related.

In summary, it is found that both nitrogen and protein concentrations of the helminth extracts are low. Nevertheless when injected into rabbits these dilute antigens are sufficient to produce antisera having moderately high titers. The antisera react more strongly with their homologous antigens than with any heterologous antigens containing an equivalent nitrogen content. The intensity of the precipitin tests parallel in general the systematic position of the species tested, although the principle of reciprocal relationship does not hold. An exception to this parallelism is of interest in that the Acanthocephala reactions indicate a closer affinity to the Platyhelminthes than to the Nemathelminthes.

8959 P

Production of Carcinoma of the Uterus in Mice.

ISABELLA H. PERRY. (Introduced by C. L. Connor.)

From the Department of Pathology, University of California Medical School.

Spayed and normal young adult female mice were treated twice a week with a .3% solution of 1:2:5:6 dibenzanthracene and a 0.1% solution of estrone* painted on the nape of the neck. Benzene was the solvent for both substances. Treatment with estrone was begun 9 weeks after the treatment with 1:2:5:6 dibenzanthracene because of its more rapid action. Twelve weeks after the treatment with estrone was begun the dose was cut in half because of the development of pyometria. The original dose was estimated to be 125 R.U. Treatment was continued throughout the life of the animals. Six months after the 1:2:5:6 dibenzanthracene treatment was begun there were 27 mice in the group when a mouse died with a large epidermoid carcinoma of the cervix. In the tenth month of the experiment 2 other cases of epidermoid carcinoma of the cervix occurred. These were in the last mouse surviving in the spayed and in the normal group. Both of these mice built nests persistently in the last weeks. All of the animals developed marked hyperplastic, cystic, and metaplastic changes in the breast and uterus. Forty-three percent of the colony developed carcinoma of the breast. Two of the mice developing carcinoma of the cervix had carcinomas of the breast and pyometria. Pyometria and car-

* The estrone was generously supplied by Parke, Davis & Company through the courtesy of Dr. Oliver Kamm.