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A Complement-fixing Antibody in Sera of Rabbits Bearing Brown-Pearce Carcinoma.

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Kidd[1] and Dmochowski[2] independently have reported the demonstration of a serologically active substance associated with extracts

1 Kidd, J. G., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 292.

2 Dmochowski, L., *Compt. rendue de la Soc. de Biol.*, 1938, **129**, 349.

of the Brown-Pearce carcinoma.[3] Recently Kidd[4] has demonstrated the immunological specificity of the material and has outlined its more important physical properties. The analogy to the serologically active substance extracted from virus-induced rabbit papillomas is striking.[5] The Brown-Pearce carcinoma, however, resembles most mammalian tumors in that the immediate cause of the proliferative process is unknown; in particular no virus agent has been demonstrated. Thus Kidd's observations seemed sufficiently important to justify the repetition of his original serological experiments.

Materials and Methods. The tumor was obtained through the kindness of Dr. J. B. Murphy in March, 1939. It was transplanted through a series of normal hybrid adult rabbits obtained from several dealers. The intratesticular route of inoculation was used. The material was obtained from vigorously growing testicular tumors or from metastases and was prepared essentially in the manner originally described by Brown and Pearce[3] with minor modifications. Recognizable takes developed in 78% of the cases. A few animals were inoculated simultaneously by several routes—intraperitoneal, intratesticular and intramuscular—in order to insure large masses of tumor developing at the same time. Another small group was immunized by Besredka's method of intracutaneous inoculation.[6] The animals were bled and sacrificed at some time between the 9th and 182nd days after inoculation. The tumor material was removed under aseptic precautions, freed of grossly necrotic elements and stored in Petri dishes at –20°C or under 50% glycerol-Locke's solution at 4°C according to the method outlined by Kidd.[4] Pathological sections were made of any grossly atypical tumor material before it was accepted as being a satisfactory antigen. Kidd's[4] directions were followed for the final preparation. The tissue was ground in a mortar with the gradual addition of isotonic saline to make a 1:20 suspension, permitted to stand overnight in the refrigerator and then spun twice at 3800 R.P.M. for 5 and 20 minutes respectively. The resulting slightly opalescent suspensions were inactivated for 30 minutes at 56°C immediately prior to use, as were the sera, which were obtained sterilely and stored without preservatives in the refrigerator. The actual test was set up using 0.2 cc of 1:4 dilution of serum, 0.2 cc of complement containing 2 units and finally 0.2 cc of a 1:20 suspension of the antigen, the

[3] Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1923, **37**, 601.

[4] Kidd, J. G., *J. Exp. Med.*, 1940, **71**, 335 and 351.

[5] Kidd, J. G., *J. Bact.*, 1940, **39**, 349.

[6] Besredka, A., *Ann. Inst. Pasteur*, 1936, **56**, 125.

materials being added in this order. Following a period of 2 hours at room temperature to permit the fixation of complement, 0.4 cc of sensitized red cells were added, the tubes shaken and placed in a 37°C waterbath. Readings were taken at 15 minutes, 30 minutes, and 60 minutes, and a final one after the tubes had been permitted to stand at room temperature overnight. In practically all instances the significant changes had occurred at the end of 30 minutes. In every case anticomplementary controls were set up. None of the antigens showed anticomplementary effects even in 1:10 dilution (*i.e.,* twice the amount used in the actual test). Several sera had to be discarded, however, since non-specific fixation could be demonstrated when a 1:4 dilution of the serum was used.

Results. The sera of 35 rabbits bearing tumors at the time of bleeding as proven by immediate autopsy were tested; of these 30 gave negative results. A number of the specimens of sera were run against more than one antigen, a total of 101 tests being recorded. Five sera definitely fixed complement on one occasion at least. When each was tested against several antigens the results shown in Table I were obtained.

The clinical state of the 5 rabbits furnishing positive sera is summarized in Table II.

TABLE I.
Antigens.

Serum	1	2	3	4	5	6	7	8	9	10	11	12	Positive	Negative	% Positive
201	+	0	0	0	—	0	—	0	—	+	+	—	3	5	37.5
352	+	+	+	+	—	0	—	—	—	—	0	+	5	2	71
361	0	0	+	0	—	0	—	—	—	—	0	—	1	5	17
473	—	+	+	0	0	0		—	—	—	0	—	3	4	43
498	0	0	+	0	0	0	0	—	0	—	0	—	1	8	11
Totals													13	24	35

+ fixation of complement.
0 no fixation of complement.
— not tested.

Antigens	Rabbit No.	Tumor
1	103	frozen metastatic
2	107	glycerolated metastatic
3	107	" testicular
4	201	" "
5	315	frozen metastatic
6	315	" testicular
7	327	" "
8	400	" metastatic
9	400	" testicular
10	437	glycerolated testicular
11	471	" "
12	473	frozen metastatic

TABLE II.

Rabbit	Days elapsed since inoculation	Clinical and pathological notes
201	31	Animal received multiple intratesticular, intraperitoneal and intramuscular inoculations. Animal beginning to lose weight. Tumors present at all inoculation sites plus widespread visceral and pulmonary metastases. Marked central necrosis in original growths.
352	36	Animal received bilateral intratesticular inoculations. In good condition at time of autopsy. Tumor limited to left testicle which showed advanced central necrosis with considerable surrounding inflammatory reaction.
361	35	Animal received bilateral intratesticular inoculations. Clinical condition poor with obvious weight loss and paralysis of hind quarters. Autopsy showed bilateral testicular involvement with the usual amount of central necrosis, and small omental and bilateral renal metastases.
473	26	Animal received bilateral intratesticular and intramuscular inoculations. Clinical condition poor with definite weight loss. Autopsy revealed typical take in left testes with many metastatic nodules of small size in the peritoneal cavity.
498	37	Animal received bilateral intratesticular inoculations. Clinical condition apparently good at time of being sacrificed. Autopsy showed bilateral testicular involvement with widespread abdominal and pulmonary metastases.

In the non-tumor bearing groups completely negative results were obtained throughout. The sera of 4 animals in whom clinical takes had recently regressed gave no evidence of complement fixation when tested against 3 known positive antigens. Specimens from 12 rabbits refractory to persistent intratesticular inoculations of tumor material failed to fix complement as did the sera of animals immunized by Besredka's method[6] (intracutaneous inoculation of the tumor followed by regression and the development of immunity against subsequent intratesticular inoculation of fresh neoplastic material).

Controls with normal sera were run with each antigen tested. Forty such tests were set up using specimens obtained from 16 rabbits uninoculated with the Brown-Pearce carcinoma. No specific fixation of complement was observed in any of the tests performed.

An attempt was made to ascertain whether the difficulty in securing positive results lay in the sera or in the antigens used. Dr. Kidd very kindly furnished 6 specimens of serum as follows:

Specimens 1, 2, 3 from domestic rabbits 5-01, 5-04, and 5-08 withdrawn before inoculation of tumor material.

Specimens 4, 5, 6 from domestic rabbits 5-01, 5-04, and 5-08,

TABLE III.

Serum	Positive reactions	Negative reactions
1	0	6
2	0	3
3	0	5
4	6	0
5	3	0
6	5	0

obtained 47, 54, and 47 days respectively after inoculation of the tumor. These 3 specimens had given consistently positive reactions in Dr. Kidd's hands. They were tried against the following antigens prepared by the author: Frozen metastases, 2 specimens; fresh metastases, 1 specimen; frozen testes, 3 specimens; glycerolated testes, 3 specimens.

The results are given in Table III.

It appeared, therefore, that the tumor extracts used in the present series of experiments did contain a substance which fixed complement in the presence of sera of rabbits bearing Brown-Pearce carcinoma growths used by Dr. Kidd in his original work.

Discussion. Among the sera of 35 rabbits bearing Brown-Pearce carcinoma in varying stages of progression and retrogression it has been possible to demonstrate the presence of complement fixing antibodies in only 5 specimens. Kidd's work has been confirmed, but the percentage of positively reacting sera is much lower in the present series than in that originally reported by him (14% as opposed to 80%). If it be assumed that no technical error invalidated the results of the test, the explanation for these divergent findings must be sought either in differences in the animals, in the time of bleeding, in the amount of tumor injected, or in the nature of the various antigenic preparations.

The animals used in the present series were a hybrid lot in contrast to the relatively more homogeneous group studied at the Rockefeller Institute. It is possible that the more frequent failure of the former group to develop antibodies is due to individual differences between the rabbits used.

Our animals were bled once—*i.e.*, just before being sacrificed. Since Kidd[4] has shown that rabbits may develop antibodies quite suddenly only to lose them rapidly within the course of 10 days, it is possible that a systematic bleeding at weekly intervals might have led to the discovery of more positively reacting sera.

Kidd's animals were given intramuscular as well as intratesticular inoculations. In nearly all cases those of the present series received only the latter. Possibly a greater antigenic stimulation resulted

from Kidd's procedure. That this is an unlikely factor is suggested by observations that the amount and state of the tumor tissue appeared to bear no definite relation to the presence or absence of the specifically acting antibodies. Our data indicated that the presence of antibody was often associated with the existence of spreading metastases, although 15 rabbits with metastases failed to show the specific antibody.

In a personal communication to the author Kidd has stated that no difficulty had been experienced in securing suitable antigenic material from any specimen of Brown-Pearce carcinoma provided that the tissue was not entirely necrotic. The antigens used in the present series gave positive reactions with at least one serum (Rockefeller or Harvard) but the possibility of the existence of quantitative differences was suggested by the fact in no case did any of the author's sera give positive reactions with all the antigens (Table I). Thus the possibility exists that saline extracts of various tumors may vary in their capacity to fix complement in the presence of sera containing the specific antibody.

Summary. In confirmation of Kidd's work it has been demonstrated that a substance associated with the Brown-Pearce carcinoma fixes complement in the presence of serum of some rabbits bearing the specific tumor. But an incidence of positive reactors considerably lower than that reported by Kidd was observed. Several possible explanations for this discrepancy are discussed.

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Biological Properties of Pregneninolone (17-Ethinyl Testosterone) in Women.

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Inhoffen, Longemann and Serini[1] have synthesized a compound (Δ^4 pregnenin-20-on-3-ol-17; also known as pregneninolone, 17-ethinyl testosterone; anhydro-hydroxy-progesterone) which possesses an unusual variety of biological properties.

This compound, which is chemically very closely related to testosterone and progesterone, has been shown to have: (a) a pro-

[1] Inhoffen, H. H., Longemann, W., and Serini, A., *Ber. Deutsch. chem. Ges.*, 1938, **71**, 1024.