radioactive elements by prefixing "ra-" before the ordinary symbol. Mr. Petty seems to base this on the supposition that Ra is the correct prefix for the symbol of the natural radioactive elements such as Radiothorium, etc.

In order to avoid confusion, I should like to point out that the best usage has never employed Ra in this way as a prefix for "radio" but Rd, as for example, Rd Th, Rd Ac, etc. All the advantages of Mr. Petty's proposal may be retained and uniformity conserved if we continue to use Rd for the natural radioactive elements and use "rd-" for the artificial ones.

In case there is more than one active isotope of an element, we shall still have to give the mass number as a superfix. In case of isobaric isotopes (for the existence of which additional evidence is accumulating), we shall also have to indicate their life periods in parenthesis or adopt some other convention.

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SPECIAL ARTICLES

THE TRANSMISSIBLE AGENT IN THE ROUS CHICKEN SARCOMA NO. 1[1]

DURING the past decade an impressive amount of evidence has been accumulated associating the lipids, and more particularly the sterols, with growth of tissue, both abnormal and physiological.[2] The chemical relationship between the estrogenic hormones, many carcinogenic substances and cholesterol has been reported and reviewed by a large number of workers. While this investigation was in progress, Claude reported the partial purification of the Rous sarcoma filtrate. A large proportion of the protein and carbohydrate was removed by indirect means, leaving an active fraction which was largely lipid in nature. The lipid fraction was separated more completely by use of acetone, alcohol and ether, but no mention is made of its activity.[3]

The methods applied in this laboratory were aimed directly at isolation of the lipids, the usual chemical procedures being altered according to the known frailties of the agent, which are primarily those of thermolability and spontaneous oxidation. The first procedures yielded a product less active than one obtained by subsequent modifications but is worthy of note because of the additional information regarding solvents which can be utilized in the study of the active substance.

Between 200 and 400 grams of fresh tumor tissue, obtained by routine transmission of the Rous chicken sarcoma in Rhode Island Red chickens, was ground in a meat grinder and shaken from three quarters to one hour in an equal quantity of acetone. The acetone extract was separated by centrifugation and filtered. It was then concentrated *in vacuo,* under nitrogen, at 37° C. until only the watery residue remained, and injected immediately into chickens as the first acetone fraction. The partially dehydrated tumor was ground in a mortar with a second portion of acetone and similarly shaken, centrifuged and filtered clear. This, after evaporation of the solvent, was injected as the second acetone fraction. At times the first and second acetone fractions were combined and part injected as the combined acetone fraction. The remainder was re-extracted with benzene or, at a later date, carbon tetrachloride by shaking with several changes of the specific solvent. Meanwhile the acetone-treated tumor was extracted for three to four hours in a Soxhlet in partial vacuum at approximately 37° C. with either benzene or carbon tetrachloride. This was combined with the benzene or carbon tetrachloride extract of the acetone fraction, filtered clear and the solvent removed in vacuo as above. The residue was dissolved in benzoinated lard at 37° C. or suspended in saline and injected into the breast of chickens. Further details of this and subsequent procedures, as well as analytical studies carried out on these fractions will be described later *in extenso.*

Tumors identical with the Rous chicken sarcoma in morphology, metastatic habits and ease of transmission were obtained by injection of the first and the combined acetone fractions. The animals were given repeated inoculations at weekly intervals after the custom of those working with the synthetic carcinogenic substances. The earliest tumors to appear followed three injections. The majority developed tumors only after an interval of three to four months. The second acetone fraction and the benzene or carbon tetrachloride extract were unsuccessful when injected alone. The residue of the tumor after having been dried and in good part freed of the lipids did not produce tumors.

Feeling that the specific cancerogenic substance might still be present in the more purified benzene

[1] From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City. This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

[2] L. Loeb, *Jour. Am. Med. Asn.,* 104: 1597, 1935; A. Lacassagne, *Am. Jour. Cancer,* 27: 217, 1936; D. A. MacFayden and E. Sturm, SCIENCE, 84: 49, 1936; F. Breinl and E. Chrobok, *Ztschr. f. Immunitäts.,* 86: 274, 1935.

[3] A. Claude, *Jour. Exp. Med.,* 61: 27 and 41, 1935.

extract but in a form too readily inactivated in the body, the extract was prepared as above and treated as follows: The breast of a normal chicken was inoculated repeatedly with kieselguhr, which is known to stimulate a mononuclear and giant cell response. After two days the yellow granular exudate thus produced, together with a small amount of muscle, was scraped out and a 10 per cent. suspension prepared by grinding with sand in normal saline. The suspension was centrifuged at high speed and the supernatant fluid separated. The benzene extract of the tumor which had been evaporated to a sticky mass was then suspended in this saline extract of normal chicken wandering cells and muscle, incubated one hour under nitrogen at 37° C. and injected immediately. Large tumors with many metastases were produced, and these were capable of transmission for an indefinite period. Again repeated inoculations were required.

Subsequent studies were aimed at the isolation of a lipid fraction, by methods less injurious to the active agent. The acetone technique was discarded and the ground tumor tissue was frozen with carbon dioxide snow and desiccated in the Flosdorf-Mudd Lyophile apparatus. The dried material thus prepared shows no loss of activity. Upon removal from the high vacuum apparatus, the dried tumor was immediately extracted with benzene, or, at a later date, with carbon tetrachloride, in a Soxhlet as before. This extract gave negative results if injected alone, but when incubated with a saline extract of chicken muscle prepared as above, produced large tumors more rapidly than those hitherto obtained. This experiment has been repeated on several occasions during the past year, since the procedure was utilized as a control for further studies, and the extract thus prepared has been found capable of producing tumors within two to three weeks following a single injection. Other chickens were given two or three injections. Almost 100 per cent. of the chickens developed tumors, and the majority appeared to be highly malignant.

The residue of dried tumor after extraction with benzene still retained a degree of activity, which was to be expected, since extraction with benzene or carbon tetrachloride is less complete if the tissues have been previously frozen and dried than if acetone is used as the dehydrating agent. The disadvantage of the material waste was more than compensated for by the superior activity of our final fraction.

A series of animals was subjected to a slight modification of procedure. Kieselguhr was inoculated into the breast muscle several times until considerable injury had occurred, and the lipid extract of the tumor was then injected alone. Tumors did not develop, showing that a period of contact with normal tissue extract *in vitro* is necessary for the production of tumors.

To test the possibility that the active agent was a contaminant introduced by contact with instruments or glassware used in the routine transplantation of the tumor or that a ubiquitous agent existed, capable of acting upon tissues prepared by the inoculation of these lipid fractions, the same procedure was followed, using the lipid fraction of normal chicken muscle. This extract obtained after freezing, drying and extraction with carbon tetrachloride failed to produce tumors alone or following incubation with a saline extract of muscle. The same precautions as to temperature and the use of nitrogen were observed throughout the extraction, and the material was injected immediately. Repeated inoculations were given and the animals kept for an equal period of time.

The lipid extract of the Rous chicken sarcoma No. 1, then, obtained by use of a specific solvent at low temperature under relative anaerobiasis, was capable of transmitting the tumor to chickens when allowed contact with chicken tissue extract for a brief period and inoculated promptly. Tumors did not develop if the lipid extract was inoculated alone or into previously injured breast muscle.

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SOME EFFECTS OF ANDROGENIC SUBSTANCES IN THE RAT[1]

A. THE EFFECT OF MALE HORMONE EXTRACTS ON THE TESTES OF HYPOPHYSECTOMIZED RATS

WALSH, Cuyler and McCullagh[2] reported that the daily administration of extracts of male hormone prepared from urine would prevent the usual atrophy of the testes that occurs following hypophysectomy in the rat. In their experiments injections of male hormone (9.0 B.U. per day) were instituted the day after hypophysectomy and continued for three weeks. To our knowledge this amazing finding has never been confirmed or denied.

We have recently made a series of experiments in which a comparison has been made between the effects of castration and hypophysectomy on the reproductive system of male rats (4 to 5 months old). As an extension of that study it has seemed desirable to compare the effects of male hormone extracts on the reproductive systems of such animals. The maintenance of the various sex accessory structures has

[1] These investigations were aided by grants from the Anna Fuller Foundation, the Fluid Research Fund of Yale University, and the Committee for Research in Problems of Sex of the National Research Council and the Rockefeller Foundation.

[2] E. L. Walsh, W. K. Cuyler and D. R. McCullagh, *Am. Jour. Physiol.*, 107: 508, 1934.