A PRELIMINARY NOTE ON THE EXTRACTION OF A CARCINOGENIC FACTOR FROM PRIMARY HUMAN MAMMARY CANCER[1]

THE following are the preliminary results of an experiment in which extracts of two human mammary cancers produced tumors at the site of injection in experimental animals.

In reviewing the extensive work that has been done on the hydrocarbon carcinogenic agents, their chemical and physical properties and relation to the normal sterols of the animal body, the common property of the fat solubility seems to stand out as a characteristic that might also be common to a hypothetical human carcinogenic factor. If such a factor exists, it would likely be extractable by fat solvents, and the site of an early primary growth would serve as an indicator of the region of greatest concentration locally. Since the solubility of the factor was unpredictable, and it seemed likely that it would be present in minute quantity, the use of several solvents would increase the chance of complete extraction.

With the above assumptions in mind the following experiment was performed. On August 23, 1939, a small, 2 cm × 2.5 cm, scirrhous type carcinoma with axillary node involvement was removed by right radical mastectomy. After small blocks of tissue had been excised for fixation and microscopical examination, the primary nodule with its immediately surrounding fatty tissue was dissected free of the remainder, ground to a hash, covered with cold acetone and stored in a refrigerator. Acetone was used to serve both as a solvent and as a dehydrating agent to prepare the tissue for later ether extractions. The material was then placed in a Soxhlet extractor and extracted exhaustively at the lowest possible temperature with the following solvents in this order: 1. acetone, 2. ethyl ether, 3. petroleum ether (B.p. 35–60° C.), 4. absolute ethyl alcohol. In an attempt to minimize chemical change, this order was maintained to remove as much fat soluble matter as possible before the higher temperature of the alcohol extraction. Separate portions of the hash were then each extracted with each solvent for at least 24 hours. The solvents were then removed by distillation at reduced pressure. The fractions were then combined for injection into experimental animals, advantage having been taken of the neutral fat as a solvent. This extract was designated H.M.Ca. Extract No. 1.

On October 10, 1939, a second human scirrhous carcinoma was obtained following a left radical mastectomy. The primary nodule measured 4.5 cm × 4.5 cm × 1.5 cm and metastatic cancer involved the axillary nodes. This tumor was extracted by an identical procedure and the final extract was designated H.M.Ca. Extract No. 2.

The experimental animals used were virgin female mice one month old of Little's C57 black low tumor strain. The strain has been maintained pure by brother-sister mating only and no spontaneous tumors have occurred in 200 control animals.

On September 12, 1939, 0.2 cc of H.M.Ca. Extract No. 1 was injected subcutaneously into the region of the left hind-most breast of 4 virgin females. Subsequent injections of 0.2 cc, alternating left and right hind-most breast regions, were given on October 11, 1939, November 21, 1939 and December 4, 1939. To avoid any continuous effect of whatever estrogenic substance the extract might contain, no further injections were given. These animals were each allowed a single pregnancy during the injection period. No animal was injected during lactation.

H.M.Ca. Extract No. 2 was first injected into the region of the left hind-most breast of 5 virgin females on November 17, 1939. One subsequent injection was given in the region of the opposite breast on December 3, 1939. All of these animals were kept virgin throughout the experiment.

The first tumor appeared on June 20, 1940, 282 days after the first injection of H.M.Ca. Extract No. 1. A hard subcutaneous nodule 0.5 cm in diameter was found at the very site of the first injection. This mass grew rapidly and on July 1, 1940, had reached 2 cm × 2.5 cm in size. The animal was sacrificed and a portion of the tumor was transplanted into 4 mice of the same strain. After microscopical preparation of the remainder, the tumor was found to be a rapidly growing spindle cell sarcoma. No gross metastases were found, but microscopically the mass extensively invaded the abdominal wall. The transplants grew rapidly in all of the animals and resulted in a 2 cm ulcerating mass by the twentieth day. Three of the animals died from the effects of the transplant between the twentieth and twenty-sixth day. On the twenty-sixth day the fourth animal, obviously dying, was sacrificed and the tumor was retransplanted. In each case an equally large internal mass was found in the abdominal cavity where the subcutaneous transplant had invaded the abdominal wall. No gross metastases were found in other regions of the body.

On July 3, 1940, 229 days following the first injection of H.M.Ca. Extract No. 2, a tumor appeared in this series of animals. This tumor also appeared at the very site of the first injection. It was allowed to grow until July 22, 1940, when it had reached 1.5 cm in size. A portion of this was transplanted into 4 animals and grew rapidly. Microscopical examination again revealed a spindle cell sarcoma. The mass had invaded the muscle to which it was adherent, but no gross metastases were found.

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To date, no other tumors have appeared in either series of animals.

Apparently, for the first time, a factor has been extracted from primary human cancer which is capable of producing tumors in an experimental animal. Further trials of the above experiment are now under way, in addition to the obvious control experiments that will be necessary to establish this finding. Also, an attempt is being made to isolate the active factor from the pooled extracts of several human cancers. These results will be reported when they are completed.

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INSECT LIFE WITHOUT VITAMIN A

IN a series of earlier studies[1] it was found that *Blattela germanica* L., the ordinary cockroach, could grow to maturity upon a synthetic diet of purified casein, starch, salt mixture and yeast or yeast extract. Since this diet was very low in vitamin A, this species must either have synthesized this factor or have had no need for it. Inasmuch as vitamin A seems essential for all the higher vertebrates that have been studied, it is interesting that it may play no part in the life of one or possibly many species of insects.

A new series of experiments was devised to check the earlier results showing no dietary need for this vitamin. The work was then extended still further to determine whether or not the cockroach could synthesize this factor within its body when fed diets devoid of vitamin A or its precursor carotene. The stock diet that has been in use for many years by us for producing cockroaches is a mixture of equal parts of whole wheat flour and dried skimmed milk. This diet was exposed to hot air for six hours at 115° C. to destroy any carotene. The young cockroaches, started two days after emerging, grew better upon this heat-treated diet than upon the original. The purified diet deficient in vitamin A and used in the usual assay procedure for vitamin A was then tested as a stock diet. All these studies indicated that the cockroach could thrive upon diets that are so deficient in vitamin A that they will not support the growth of rats.

The next step to determine if this insect could carry on its body functions without vitamin A consisted in producing large numbers of the insects upon an A-free diet, extracting the fat from these insects and testing this fat for this vitamin.

By the use of large cages 2.5 kilograms of live cockroaches were produced in the course of ten months. These were reared upon the vitamin A-free diet used in the U. S. P. method for the vitamin assay with rats. From these insects were extracted very carefully in the cold 150 grams of oil. This oil was tested colorimetrically for vitamin A but gave only a negative test. It was then fed to rats in accordance with the usual procedure for the assay of vitamin A. Levels of 0.1 and 0.01 grams were fed daily in this assay. Neither level gave any indication of containing vitamin A. In the same assay the reference cod liver oil gave the usual response in growth and prevention of eye symptoms.

From these results it is evident that the cockroach needs no vitamin A in its diet and that its body can function normally throughout its life cycle without this vitamin. Therefore vitamin A is not of universal importance in the life of animals.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A DIFFERENTIAL METAL BELLOWS MANOMETER FOR THE MEASUREMENT OF BLOOD FLOW

THE differential rubber membrane manometer described in an earlier report for the measurement of blood flow by differential manometry[1] has been replaced by a pair of metal bellows manometers, arranged to record mechanically the difference in their pressure readings. The calibration of the manometer couple in this arrangement has remained constant over a period of eight months in almost daily use.

The bellows is a deeply corrugated thin-walled cylinder, which elongates under application of internal pressure.[2] The most flexible small bellows obtainable has an outside diameter of 25 mm and a length of 30 mm. Without load, it responds to internal pressure with elongation at the rate of approximately 0.04 mm for 1 mm Hg in roughly linear fashion up to at least 200 mm Hg. To adapt the bellows for differential manometry, a pair of them was mounted on suitable bases (Fig. 1, *a*) and clamped in position with their movable faces apposed so that each bellows exerted its full thrust against the other. Magnified mechanical recording of the movement at the apposed faces was accomplished by inserting between the faces a short sleeve (Fig. 1, *b*) bearing a rod onto which the short arm of a recording lever was slotted (Fig. 1, *c*). The

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[1] C. M. McCay, *Physiol. Zool.*, 11, 89, 1938.
[1] Hampden Lawson and J. P. Holt, *Jour. Lab. and Clin. Med.*, 24: 639, 1939.

[2] The courtesy of the Fulton Sylphon Company, Knoxville, Tenn., who supplied the bellows from special stock, is gratefully acknowledged.