Abnormalities in the Distribution of Biotin in Certain Tumors and Embryo Tissues

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In a preliminary note (13) it was pointed out that certain tumors contained much more, others significantly less, vitamin H or biotin than did normal adult tissues. It was also observed that embryo tissues deviated from the normal biotin levels in the same manner as the corresponding tumors. Because of the indispensability of biotin for the growth of many microorganisms and higher animals it was considered possible that these differences might reflect fundamental metabolic changes characteristic of rapidly multiplying cells. Therefore, the earlier observations have been extended to include many more tumor types. In addition, various attempts have been made to alter the biotin content of tumors experimentally and thus influence their growth.

MATERIALS AND METHODS

Tissues were assayed for biotin by a new microbiological method using the indicator organism *Rhizobium trifolii* 205, for which biotin is a specific growth factor (12). Only a small proportion of the biotin in tissues is in free form (Table I), the remainder being firmly bound and liberated from such combination only after hydrolysis. Thus the treatment of the tissue to be assayed differed somewhat depending upon whether free or total biotin was to be determined.

For total biotin assays, about 0.3 to 0.5 gm. of tissue was extracted by autoclaving with 5 ml. of 10 per cent sulfuric acid for one hour at 15 pounds' pressure which freed the biotin from its combination in the tissues. The extract was then filtered into a calibrated tube, the solid debris washed, and washings added to the filtrate. After neutralizing the filtrate to bromthymol blue with 25 per cent sodium hydroxide it was diluted to 10 ml.

For free biotin assays, acid hydrolysis was of course not necessary. A similar amount of tissue was simply chopped very finely and extracted with 5 ml. of water by placing the tubes in a boiling water bath for 20 minutes. The mixture was then filtered, the residue washed as before, and the filtrate diluted to 5 or 10 ml.

The basal biotin-free culture medium for the test organisms consisted of the following: mannite 5 gm., dipotassium phosphate 0.5 gm., potassium nitrate 0.2 gm., magnesium sulfate 0.2 gm., sodium chloride 0.1 gm., calcium sulfate 0.1 gm., ferric chloride 0.01 gm., thiamin 100 γ, β-alanine 100 γ, distilled water 1 liter. The mixture was brought to boiling, filtered while hot, and on cooling adjusted if necessary to pH 6.8. This medium is incapable of supporting growth of the organism, but since the only additional factor required is biotin, growth occurs upon the addition of tissue extracts in proportion to the amount of biotin they contain.

Various dilutions of the tissue extract to be assayed were measured in amounts from 0.2 ml. to 0.001 ml. into 125 ml. Erlenmeyer flasks, and 25 ml. of basal medium then added to each. After autoclaving for 30 minutes at 15 pounds' pressure, these mixtures were inoculated with one loopful of a light suspension of bacteria in sterile distilled water (approximately 50 to 100 million cells of *Rhizobium* per ml.). The inoculum was grown on slant cultures consisting of basal medium plus agar 1.5 per cent and a small amount of biotin supplied in the form of 0.1 per cent Difco yeast extract. Insufficient biotin is carried in such an inoculum to permit growth in the basal medium.

These inoculated mixtures were incubated for 5 days at 28° C., after which the opacity resulting from bacterial growth was measured with the Evelyn photometric colorimeter, filter 540. From standard curves for this organism, readings were converted into numbers of *Rhizobium* per ml. and the results expressed graphically. By comparison with crystalline biotin or standardized yeast extract curves included in every assay, the amount of unknown extract inducing half-maximum growth was determined, and since this corresponds to the activity of 0.32 millimicrograms of biotin in 25 ml. of medium, the biotin content of the extract could be readily calculated. All results are expressed in mg (1 X 10⁻⁹ gm.) of biotin per gm. of dried tissue.
THE BIOTIN CONTENT OF TUMOR, EMBRYO, AND NORMAL ADULT TISSUES

In this comparative study of the biotin contents of normal and malignant tissues considerable care was exercised in the selection of material. Only those tumors for which suitable control tissues could be obtained were chosen. Samples of each were examined microscopically and unless found to be quite healthy and reasonably free from other tissues, the material was discarded. It is obvious that these restrictions must have eliminated at once a considerable proportion of all available human material and much animal material as well. For example, many of the commonest tumors, such as carcinoma of the breast or uterus, were unsuitable because a control sample of the corresponding normal epithelium was not available. Likewise carcinomas of the stomach or intestine were usually valueless, either because of necrosis in the tumor itself or of inability to obtain normal control epithelium free from underlying tissues. The results which are reported below, therefore, represent as nearly as possible a true comparison of the vitamin H contents of certain normal tissues and their malignant derivatives.

**Skin tumors.**—Many specimens of the Shope rabbit papilloma and related carcinomas were generously supplied for this work by Dr. John G. Kidd, of the Rockefeller Institute for Medical Research. By careful dissection it is possible to obtain rabbit skin relatively free from the subjacent connective tissue, so that by microscopic comparison there is an equally high proportion of epithelial tissue in both normal skin and tumor. Results of biotin assays (Fig. 1) indicated an average of 360 μg of biotin per gm. of tissue for 5 papillomas, while 16 skin carcinomas averaged 369 μg. In contrast to this, four samples of normal skin taken from areas adjoining the papillomas contained only 89 μg of biotin per gm. Since certain similarities in the carbohydrate metabolism of embryonic and tumor tissues are known to exist, del-

![Fig. 1](image-url)

Fig. 1.—A comparison of the biotin contents of certain tumors with analogous normal adult and embryo tissues.

determinations of biotin in the former were included for comparative purposes whenever possible. The skins of domestic rabbit fetuses approximately 3 days before birth contained an average of 531 μg of biotin per gm. From these assays it is apparent that although skin papillomas and carcinomas of the rabbit do not differ significantly in biotin content, they are both far richer in the vitamin than the corresponding normal adult tissue, while the value for embryo rabbit skin is still higher.

**Liver tumors.**—Primary tumors of the liver, when found to be well circumscribed and free from necrosis and connective tissue, were considered ideal for biochemical study, since an adequate supply of normal liver was readily available from the same animal for comparison. Such tumors were produced experi-
mentally in Wistar rats by feeding a diet of unpolished rice plus 0.06 per cent p-dimethylaminobenzene (butter yellow) over a period of 3 to 6 months. Several suitable tumors were obtained, 6 of which on analysis were found to contain an average of 2900 mg of biotin per gm. (Fig. 1). Some had considerably less biotin than this, but were eliminated because histological study showed that they contained excessive amounts of connective tissue. Parts of the tumor-bearing livers which were shown microscopically to be free of cirrhosis as well as of tumor, were used as controls, and averaged 4480 mg biotin per gm., a value almost identical with those obtained for the livers of normal nonpregnant adult Wistar rats on the standard laboratory ration of equal parts Purina dog chow and Rockland mouse or rat diet, with water ad libitum. Embryo rat liver taken on the 17th to 18th day of gestation possessed only 1280 mg of biotin per gm. Here again, therefore, embryo and tumor tissue were found to differ in their biotin content from that of the normal adult tissue, although in this instance the deviation was in the opposite direction from that of the skin tumors previously discussed.

The close parallelism between the observed abnormalities in biotin level of embryo and tumor suggested that perhaps the difference in vitamin H content is related to changes normally occurring in all rapidly dividing cells rather than to any metabolic peculiarity of those particular tissues. To clarify this point, rats were partially hepatectomized, and the vigorously regenerating livers assayed for biotin 3, 7, 14, and 21 days after the operation. Even during the most active phase of regeneration, which occurred in from 3 days to one week, there was no significant change in the biotin levels, 12 regenerating livers averaging 4780 mg per gm. (range 4300 to 5720).

Similar determinations were made on a number of subcutaneously transplanted liver carcinomas of mice, which were generously supplied from the National Cancer Institute by Dr. Howard B. Andervont. The growth was originally induced by subcutaneous injections of butter yellow. It was surprising to find that in four 28-day tumors of moderate size (0.6 gm. or less) the biotin level was 3832 mg per gm. (range 3130 to 4216), practically identical with that of the control livers (from the same animals), which contained 3563 mg. The biotin levels of these livers did not differ from those of normal animals of the same strain. On assaying the carcinoma at a later stage of growth (over 1 gm.) it was found that while 6 normal livers from tumor animals averaged 3613 mg of biotin per gm., the average biotin level of nine 34-day tumors was but 1170 mg (range 555 to 2130). For comparative purposes, it may be stated that the biotin level of mouse embryo liver has been found to be 2909 mg (Fig. 1).

While these analyses of older tumors indicate changes in biotin content similar to those observed in carcinoma of the rat liver, the reason for the unchanged biotin levels of the younger mouse tumors is obscure. This discrepancy between two values for the same tumor strain has also been noted recently with the transplanted liver tumors of C3H mice, also supplied through the kindness of Doctor Andervont.

A few primary liver cell carcinomas of human beings have been assayed for biotin but there was only one from which ideal material could be obtained and for which reliable figures may be reported. This appeared as a large, firm, light yellowish-brown mass in the left lobe of the liver with several large metastases throughout the rest of the organ. The tumor contained very active tissue practically free of necrosis, while the unaffected liver appeared quite normal, with no evidence of cirrhotic change. The normal liver assayed 4450 mg of biotin per gm., and in striking contrast to this the carcinoma was found to contain only 746 mg per gm. For purposes of comparison, values for human embryo organs obtained from a 4 1/2-month fetus, indicated the biotin level of human embryo liver to be 4000 mg per gm., which, unlike that for embryo rat livers, is only slightly below adult values (Fig. 1).

**Lung tumors.**—Several strain A mice bearing the transplanted lung tumor F were generously supplied by Doctor Andervont. This carcinoma arose spontaneously and its source is thought to have been the alveolar epithelium. If so, the normal lung tissue selected as control should provide a fair, though admittedly imperfect, basis for comparison. As indicated in Fig. 1, normal adult mouse lung contained an average of 2285 mg of biotin per gm., while the carcinomas were all much lower (1477 mg). Samples of mouse embryo lung obtained near term averaged 1330 mg of biotin, a deviation from the adult value which closely approximates that of the tumors.

**Connective tissue tumors.**—Several types of transplantable sarcomas were readily available in experimental animals, and as controls normal connective tissue scraped from beneath the epithelium of adult mice was employed. This assayed 1280 mg of biotin per gm., whereas the transplanted mouse sarcomas 37 and 180 averaged 556 mg and 455 mg, respectively (Fig. 1). The biotin content of the corresponding embryo tissue proved to be 312 mg per gm., a finding which again bears out the observation that tumor and embryo appear to deviate in similar fashion from the normal adult tissue.

Adult rat connective tissue contained an average of 2460 mg of biotin per gm. While, as in the mouse, samples of connective tissue were taken from subcutaneous areas apparently free from gross fat deposits it was nevertheless impossible to obtain the tissue entirely devoid of fat cells. Fat is very low.
in biotin, however (52 mg per gm. for the rat) so that the small amounts present in the control would lower the biotin level somewhat from its true value.

The R39 sarcoma was found to contain 544 mg of biotin per gm. and rat embryo connective tissue 1280 mg (Fig. 1).

Samples of two different transplantable rabbit sarcomas, RS and Kato, were obtained through the courtesy of Doctor Kidd. These assayed 290 and 813 mg biotin per gm., respectively. In contrast to the results with normal adult rat and mouse connective tissue, the same tissue in the rabbit assayed only 134 mg. Embryo rabbit connective tissue from fetuses approximately 3 days before birth averaged 1330 mg (Fig. 1). The increased biotin content of the rabbit sarcomas over those of the control adult tissues duplicates the findings for epithelial growths in this species, and the two are contrary to those generally found for rat and mouse tumors. In either case, however, the corresponding embryo tissues consistently varied from the normal biotin levels in parallel with the tumors.

**Lymphoid leukemia.**—Because of the resemblances between the leukemias and neoplasia, the biotin levels of normal lymph nodes were compared with the enlarged nodes of lymphoid leukemia. For this purpose, nodes averaging 130 7 mg (range 1300 to 1330), the leukemic nodes 1300 mg (range 1310 to 1405) biotin per gm., an insignificant difference. Thus these nodes did not exhibit the variation found in most neoplasms, but as those of other lines might, no conclusions can yet be drawn.

**Miscellaneous tumors of human origin.**—Nearly all human material obtained either at autopsy or at operation proved to be unsatisfactory in one way or another. Many bone sarcomas were assayed for biotin value, but here the mass of intercellular material, even when inorganic constituents were eliminated, made comparisons of normal tissue and tumor extremely difficult to evaluate. Results on several bronchogenic carcinomas of the lung, although the tumors were ideal in themselves, were discarded because alveolar tissue is really not an adequate control and attempts were not made to assay bronchial epithelium. Most carcinomas of the gastrointestinal tract were highly unsatisfactory either because of necrosis or hemorrhage or excessive connective tissue in the tumor, or the presence of too much connective tissue or muscle in the sample of normal mucosa selected as a control. A few, however, did prove to be acceptable following microscopic verification of the tissues assayed. The values for the tumors in each of these instances differed widely from the control determinations. Carcinomas of the stomach, sigmoid, and rectum possessed an increased biotin content, 1500, 787, and 444 mg per gm., while the respective control values were 833, 284, and 102 mg. In two cases, however, a reverse relationship was discovered, the biotin levels of carcinomas of stomach and cecum being 483 and 1440 mg while corresponding control determinations were 1700 and 2450 mg per gm. (Fig. 1). As one would expect, the results of assays on isolated human tumors, each with its own clinical and pathological peculiarities, do not present the uniform picture which is obtained in the case of a group of standard animals bearing tumors of common origin. It is not surprising, in such a random selection of material from patients, that tumors of both the biotin-rich and biotin-poor type were encountered, for even in the normal tissues a wide divergence in biotin concentration was found in different patients, probably because of their variable states of nutrition. In cases of accidental death in healthy subjects, the biotin levels of the organs were remarkably constant.

As for benign growths of the human subject, a large lipoma in the posterior cervical region and 4 uterine fibromyomas have been examined so far. None contained more biotin than the mother tissue. Thus for the lipoma the value was 35 mg as compared with 69 mg for normal adult fat. The fibroids, all of which were obtained at operation, gave such uniform figures that they may safely be regarded as characteristic of this lesion (Fig. 1). The average was 67 mg and that for the surrounding normal myometrium 70 mg per gm.

The uterine fibroid is considered by some to be a hyperplasia rather than a true neoplasm, and if this view be correct a normal biotin content was perhaps to have been expected since even actively regenerating liver showed no increase, and the myometrium in the fourth month of pregnancy also had a normal biotin content.

**The Transition from Embryonic to Adult Biotin Levels in Various Organs of the Rat**

In the preceding pages, reference has been made repeatedly to the close parallelism which seems to exist between the biotin levels of embryonic tissues and tumors. Certain embryo tissues of the rat; e.g., liver and connective tissue, contain much less vitamin H than the same adult tissues, while with other organs the situation is just reversed. One immediately wonders whether or not the transition from embryonic to adult biotin levels is an abrupt one, whether all organs are involved at once, and whether the time
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at which this transformation takes place coincides with any known changes in metabolism of the tissues concerned.

Rat fetuses were removed at varying stages of development, the age being estimated from the age-weight tables of Donaldson (4). Sufficient embryo livers could be obtained as early as 5 days before birth, kidney at 3½ days, and other organs at 1½ days. Further samples of these organs were taken for assay at the time of birth and at intervals thereafter in order to follow closely the changes in biotin activity over the period just before and after delivery. These data are summarized in Fig. 2. The rapid shift in the

distribution of biotin among the representative organs shown is most striking. Beginning a day or so before birth, those organs characterized by a higher biotin activity in the embryonic stage lose vitamin H very quickly, and by the third post-natal day the normal adult values are reached. At exactly the same time that this precipitous decline in biotin activity is occurring in some organs, liver, which is characteristically low in the embryo as compared with the adult, shows a sudden rise, and also within 3 days after birth attains normal adult levels. As indicated in Fig. 2, the situation in the case of kidney is somewhat more complex but the same general trends are evident.

As to the meaning of these drastic readjustments of biotin value in the various organs, one can at present only hazard a guess. Comparisons made between the biotin contents of the organs of a 4½-month human fetus and the organs of normal adult persons autopsied after accidental death, present the same picture with only minor modifications. These changes in vitamin H activity might well reflect a fundamental alteration in cellular metabolism occurring at the time of transition from intra-uterine to extra-uterine life. Particularly interesting in this respect is the observation of Norris, Blanchard, and Povolny (10) and of Burk (1) that the high anaerobic glycolysis of embryo liver falls to normal adult values very shortly after birth. This is correlated by them with the decrease in hematopoietic elements in the liver which takes place at this time. Since malignant change is said by some investigators to be accompanied by increased glycolysis, both aerobic and anaerobic (1), and at the same time the biotin levels of the tumors shift back toward those of the corresponding embryo tissues, a close relationship between biotin activity and the unusual carbohydrate metabolism of embryo and tumor appears to be an interesting possibility.

Growth of Transplanted Tumors in Biotin-Depleted Mice

Various attempts were made either to raise or lower the biotin contents of the tissues of experimental animals with the idea of observing the course of transplanted tumors under such altered conditions. It soon became apparent that mice are capable of tolerating enormous doses of biotin extract without any change

in the free or total biotin content of the tissues. As illustrated in Table I, mice which had received 200 rat units of SMA vitamin H concentrate per day (0.2 ml. intravenous, 0.2 ml. intraperitoneal), did not show any increase in free biotin whatever but even a slight drop below normal in some cases. Total biotin values also remained constant, the only notable exception being the kidney, where they reached more than three times the normal. Since such results were obtained by giving maximal doses of biotin concentrate, attempts

![Fig. 2](image-url)
to raise the biotin content of the tissues had to be discontinued, although it appears possible that if pure biotin were available sufficient could be administered without toxic effects to accomplish this end.

The withdrawal of biotin from the tissues of mice proved to be much simpler. Feeding a diet rich in the biotin inhibitor, avidin (5), produced early signs of deficiency after 6 weeks, severe signs after 12 weeks, and death usually at about the 16th week. For the last few weeks of life all animals appeared cachectic, most of the fur was lost, and the skin was thin, inflamed, and in places scaly. Most of the animals were blind, and many had developed a peculiar spastic gait. Others seemed to be in constant pain, as though the cutaneous sensory nerves were irritated, because they continually licked themselves. Often the lightest touch with the tip of a pencil was sufficient to induce convulsive seizures. The mice seemed to continue in about the same condition for the last few weeks of life, and then died suddenly. Histologically the internal organs showed nothing remarkable; studies on the central nervous system are in progress.

Data given in Table I indicate the extent to which the various tissues were depleted of biotin at the time of death. All the organs assayed except kidney and brain were found to have lost 80 to 90 per cent of their biotin activity. The retention of biotin by kidney and brain is emphasized by other figures obtained at the various tissues were depleted of biotin at the time of deficiency after 6 weeks, severe signs after 12 weeks, and death usually at about the 16th week. For the last few weeks of life all animals appeared cachectic, most of the fur was lost, and the skin was thin, inflamed, and in places scaly. Most of the animals were blind, and many had developed a peculiar spastic gait. Others seemed to be in constant pain, as though the cutaneous sensory nerves were irritated, because they continually licked themselves. Often the lightest touch with the tip of a pencil was sufficient to induce convulsive seizures. The mice seemed to continue in about the same condition for the last few weeks of life, and then died suddenly. Histologically the internal organs showed nothing remarkable; studies on the central nervous system are in progress.

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As to the fate of tumors transplanted into biotin-deficient hosts, two examples will serve to illustrate. When it was estimated that a very deficient lot of animals had approximately 2 to 3 weeks to live, fragments of the rapidly growing sarcomas 37 or 180 were introduced subcutaneously into these and into normal control animals on the standard laboratory ration. These tumors, it will be recalled, are of the type which are biotin-poor in contrast to the corresponding normal tissue. Contrary to expectation all transplants grew rapidly, producing large healthy growths even though the mice during this time were in an extreme stage of biotin depletion. After 17 days the average weight of 8 sarcomas 37 from deficient animals was 0.26 gm., the average of 11 control tumors 0.36. In another group the average weight of 9 sarcomas 180 was 0.99 gm. after 10 days' growth in biotin-deficient mice in comparison with 1.06 gm. for a corresponding set of controls.

It appears therefore, that these two sarcomas, the growth of which is essentially unimpaired by extreme biotin deficiency, differ fundamentally from the normal tissues of the host, which cannot function in the absence of an adequate supply of biotin. It was at first thought that a flourishing neoplasm might rob the host of its already depleted biotin store, but on analysis it was found that the tumors contained only the same small fraction of their normal vitamin H contents as the muscle, lung, and liver of the animal in which they were growing—in this case 20 to 24 per cent.

Further evidence that these two tumors possess the power of carrying on their normal metabolic processes unimpaired by lack of a biotin supply may perhaps be furnished by in vitro studies. With the addition of avidin to a protein-free culture medium, in excess of the amount required to bind all of the available biotin (as demonstrated by inability of the biotin-requiring test organism, Rhizobium, to grow in it), the cells of sarcomas 37 and 180 continued to multiply actively. It is to be emphasized, however, that this investigation, which later will be made the subject of a separate paper, is mentioned only in passing. The growth of normal tissues in vitro in the absence of biotin has not yet been examined, as of course it must be before any significance can be attached to the preliminary observation just cited.

**DISCUSSION**

The possibility that regulatory substances present in normal cells in only catalytic amounts might be concerned in the malignant process led to this investigation. Biotin, or vitamin H, the most physiologically active vitamin known, was selected because it has been thought to be essential in the energy-yielding reactions of every normal cell.

Results have shown that if carefully selected malignant tissues be compared with their normal counterparts, striking differences in the biotin levels are found. For the most part, the tumors contained only a fraction of that amount of biotin present in the normal controls. A few types, however, were definitely higher in the vitamin. It is interesting to compare these findings with other recent investigations and to speculate upon the possible interrelationships. For example, the biotin level of liver carcinoma was found to be far below that of normal or vigorously regenerating liver, and Burk (1) has found the respiration of such tumors also to be deficient, while regenerating liver remained unchanged. Greenstein and others (6, 7) reported that the arginase and thymonucleoproteinase activity in such tumors was reduced while simple hyperplastic liver did not differ from the normal. In a recent paper (8) these investigators report similar results concerning the xanthine dehydrogenase, catalase, and amylase contents of hepatic tissues. Furthermore, they too observed a striking parallelism...
between the relative activity of fetal to adult liver tissue and that of hepatic tumor to normal liver.

The classic respiration studies of Warburg (11) and the more recent investigations of Burk and his associates (1, 2) have shown that embryo possesses certain metabolic features in common with tumors. For this reason, the biotin level of embryo tissues corresponding to the neoplasms selected was determined whenever possible, and here again, as with the tumors, it was found that all differed widely from the normal adult values. Furthermore, the deviation of both tumor and embryo was closely parallel. In other words, where the value for an embryonic organ was higher than that for its adult counterpart, the content of the tumor was high in comparison with the adult mother tissues; and where the value for an embryonic organ was lower than that for the corresponding adult structure, the tumor contained less biotin than the adult organ.

A further link between the respiratory studies of Burk (1) and the present observations is to be found in a comparison of changes occurring during the transition from embryo to adult. Immediately after birth the biotin content of the various organs of the rat undergoes rapid change and reaches the normal adult values within 3 days. Likewise, the embryo type of glucose disassimilation shifts to that characteristic of the adult very quickly after birth.

It also appears of significance that certain transplantable sarcomas were found to be capable of abundant growth seemingly independent of a supply of biotin, even though they apparently have no power to synthesize the substance. During the last week of life in animals dying from biotin deficiency these tumors were quite unimpaired by lack of this vitamin although in its relative absence the normal host tissues were unable to live.

Hitherto, the various organisms capable of growth in the absence of biotin have been shown to possess the power of synthesizing this substance themselves (9). So far as is known biotin is necessary to all living tissues and if, as is the case with certain bacteria, yeasts, fungi, and mammals, the ability to synthesize biotin has been lost, they become dependent upon an outside source for their existence. However, the two sarcomas mentioned appear to fall in a different category, for it seems that they neither synthesize this vitamin, nor depend upon an extracellular source of supply for their well-being. Perhaps this is in some way connected with the fact that even in a normal animal these tumors are biotin-poor.

The most interesting fact to emerge from the whole investigation, and perhaps the most significant, is this demonstration that the cells of the two sarcomas so far examined, unlike any other cells yet known, are almost, if not quite, independent of biotin. Should this prove true of other tumors in various species it would suggest that biotin is of little or no moment in the life of neoplastic tissues, possibly because of their altered carbohydrate metabolism, and that any shifts observed in the level of this vitamin are a consequence, and not a cause, of malignancy. Nor is other evidence lacking to support such a suggestion. For example, the biotin content of the Shope papilloma, already high, does not rise with the advent of the carcinomatous transformation. Furthermore, young transplanted hepatic carcinomas of the mouse have a biotin level similar to that of normal liver from this species, and not until they have approximately doubled in size do they show the low value that has been found in butter yellow carcinoma of the rat's liver. Yet at a time when they weigh half a gram they are just as truly carcinomas as they are when they have come to weigh one gram.

**SUMMARY**

1. With but one exception all malignant tumors studied, whether of animal or human origin, differed widely in their biotin content from the corresponding normal tissues.

2. The biotin levels of embryo tissues also diverged widely from those of normal adult tissues, the major shift from embryonic to adult biotin values occurring in the rat within 3 days after birth.

3. In all cases, the biotin content of both tumor and embryo tissues deviated in the same direction from the corresponding normal adult levels.

4. Attempts to raise the general biotin content of animals by injection of biotin concentrate were unsatisfactory because of rapid excretion of the excess vitamin.

5. Both tumors and normal tissues could be greatly depleted of biotin by maintaining the animals on a ration containing avidin, and although the animals suffered severe deficiency symptoms as a result, the growth of certain transplanted tumors was unimpaired. Thus, while biotin is known to be an essential factor in the metabolism of normal mammalian tissues, these tumors, at least, are capable of maintaining their usual activity in its relative absence.

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