INTRODUCTION

Since the discovery by Warburg (60) of the glycolytic metabolism of tumors, and the observation by Dickens and Simer (11, 13) of a lowered respiratory quotient in tumors, it has widely been assumed that they possess a metabolism differing from normal tissue, inasmuch as they combine high aerobic and anaerobic glycolysis with an R.Q. below unity.

Doubt on the significance of the aerobic glycolysis in tumors first arose when Murphy and Hawkins (44), and later Crabtree (8), found that certain new growths of the mouse had little or no aerobic glycolysis. Thus aerobic glycolysis is not invariably associated with neoplasia.

At first it was thought that with certain exceptions, which could be explained on the basis of damage caused by the conditions of measurement, normal and particularly “stationary” tissues did not glycolyze aerobically to any appreciable extent. Later, however, it was found that there are some few normal tissues which glycolyze aerobically to an extent comparable with that of the “typical” transplanted animal tumors, and therefore are much more glycolytically active than those neoplasms having little aerobic glycolysis. The retina was early recognized as such a tissue (62). Later, the medulla of the kidney, unlike its cortex, was found (28, 14) to have a glycolytic metabolism comparable in activity with the most vigorous type of tumor metabolism, and contrary to the observations of György, Keller, and Brehme (28) we found (14) that the same metabolism was shown even in the serum of the same species; evidence that this was not a case of damage due to unphysiological medium, such as is known to occur with some other tissues. We concluded (14) that this tissue—and also cartilage (15)—probably has energy requirements too great for its respiration alone to supply.

In these examples the R.Q., according to our measurements (11, 13, 14), was unity. Bywaters (7), however, found that synovial membrane glycolyzes aerobically and has a low R.Q. Its metabolic activity was of a low order, and a better example of a normal tissue with a “tumor-like” metabolism was found by us (16) in intestinal mucosa; this tissue, from the jejunum of the rat, had a glycolytic and respiratory activity comparable with that of the most vigorously growing neoplasms combined with an R.Q. of about 0.85; its aerobic glycolysis was almost equal to its anaerobic glycolysis. Evidence was presented that mechanical damage could not be held responsible.

The suggestion has therefore been advanced (3) that the high aerobic glycolysis and low R.Q. possessed by most tumors are not in reality a result of any alteration in metabolism occurring during carcinogenesis, but are referable to the fact these growths arose from normal tissues that had the same type of metabolism. This hypothesis has the merit of emphasizing the need for comparison with the tissue of origin, but unfortunately this strict comparison is possible in only a few instances since the cells of origin cannot usually be obtained in a state of purity, even when their nature is known with certainty. Further, we shall show in the Discussion that such a comparison ought to include if possible not only the adult tissue and its tumor, but also the primitive cell which may be regarded as the common ancestor of both.

Since the greatest change is to be expected when the malignant transformation is complete, it is important that the comparison with malignant as well as with nonmalignant tumors should be included in any proper investigation of this hypothesis. We be-
lieve that this point has been overlooked by Berenblum and his associates (2), who based their entire hypothesis on a single comparison between normal skin epithelium and a Shope papilloma, both of which they found to have substantially the same metabolism: No indication was given whether this papilloma had become malignant. This is a fundamental point, because it will be shown in the present paper that, while a benign tumor may have a metabolism almost identical with that of its tissue of origin, a change undoubtedly occurs in those examples studied by us when the tumor becomes malignant.

There appear to be only two other cases besides skin in which the metabolism of the tumor and that of its tissue of origin have already been directly compared. Dreyfuss (17) reported, without drawing any particular attention to the fact, an excellent example—that of human endometrium in various phases and pathological states, compared with uterine carcinoma. His results are summarized in Table I.

| Table I: Metabolism of Human Endometrium, after Dreyfuss (17) |
|-----------------|---------|---------|---------|
|                  | $\Delta Q_{O_2}$ | $\Delta Q_{CO_2}$ | $\Delta Q_{N_2}$ |
| Proliferative phase | 16.9    | 1.4     | 9.7     |
| Secretory phase    | 16.3    | 1.0     | 8.5     |
| Decidual           | 16.4    | 0.8     | 14.2    |
| Hyperplasia of endometrium | 16.5    | 1.1     | 11.2    |
| Adenomyosis *      | 16.5    | 1.2     | 10.6    |
| Carcinoma of endometrium | 13.3    | 10.0    | 14.2    |

* "The term adenomyosis of the uterus signifies the invasion of the myometrium by endometrial glands and endometrial stroma. . . . Despite this aggressive behavior, adenomyosis is clinically a benign lesion; it never produces metastases and is never fatal. . . . Its position is thus intermediate between benign and malignant growth processes."

The second example is the group of liver tumors induced by feeding azo dyestuffs to rats. Hayashi and Tomita (39) found that in rats fed aminoazotoluene the formation of adenomas and hepatomas was accompanied by a sudden increase in anaerobic glycolysis. Nakatani and his group (46), who, like all succeeding authors, including ourselves, used p-dimethylaminoazobenzene to produce neoplasms, found a gradual increase in anaerobic glycolysis during the development of the tumors; when the period corresponding roughly with malignancy was reached the aerobic glycolysis also increased notably. The remainder of the liver retained the metabolism of the precancerous phase of the dye feeding, i.e., low aerobic with high anaerobic glycolysis. Orr and Stickland (50) could not observe the latter increase of anaerobic glycolysis associated with the precancerous phase; they made the surprising observation that there was no evidence of an increase in the rate of anaerobic glycolysis of liver tissue of rats at any stage of dye feeding. Some of the normal rat livers used by Orr and Stickland had high anaerobic glycolysis, however. The most important point made by them is that in the tumors a large part, probably all, of the lactic acid is derived from glucose. In the normal adult liver this is not the case and, in fact, glycolysis is hardly increased at all by the presence of glucose (55) though it may be slightly increased in the fetal liver (54). Hence Orr and Stickland showed that a qualitative change of metabolism occurs when normal liver is transformed to tumor. Owing to scarcity of their material data on respiration, they were lacking, and those on aerobic glycolysis are solely of glucose breakdown. They showed, however, that aerobic as well as anaerobically glycolysis occurs in both cholangiomas and hepatomas.

In a preliminary account, which reached us when the present work was almost completed, Burk, Behrens, and Sugiuira (4), indicate that they alone have made a complete study of the respiration, glycolysis, and R.Q. of p-dimethylaminoazobenzene liver tumors. In general, the anaerobic and aerobic glycolysis was intermediate in amount between those of the neoplasms investigated by Warburg and of the mouse tumors studied by Murphy and Hawkins and by Crabtree. "Compared to that of the original liver tissue, however, where the anaerobic lactic acid formation is almost negligible, the increase in the hepatic tumor is relatively very marked" (4). The R.Q. of the tumors was definitely less than unity.

We have extended the observations on liver and tumors derived therefrom in two directions: (a) In addition to measuring the respiration, glycolysis, and R.Q. of hepatic neoplasms induced by p-dimethylaminoazobenzene, we have studied for comparison the benign type of hepatoma occurring spontaneously in certain strains of mice. (b) Metabolic activities other than those mentioned above have been investigated in both groups of tumors.

It was thought that a comparison of the metabolism of the neoplasm with that of adjacent liver tissue should be extended to as many component reactions as possible, since the respiratory metabolism of a cell represents the sum total of the activities of a very large number of enzymes. Two tissues may have a superficially similar respiratory metabolism, yet there may be fundamental differences in the way the metabolic structure is built up, as gains on the one side may be compensated by losses on the other. Normal and cancerous liver tissues are particularly suitable for such a comparison owing to the many highly specialized functions performed by the normal liver.

Greenstein and his collaborators (25, 26, 27) have studied various enzymes in extracts of transplanted hepatic tumor and compared them with similar extracts of normal liver. In the tumor extracts, they found reductions in the activities of catalase, arginase,
and xanthine dehydrogenase. The activity of the enzyme thymonucleosidepolymerase was only 10 per cent lower than that from normal liver, and no difference was found in the activity of "anilase." Kishi, Fujiwara, and Nakahara (34) prepared extracts from transplantable rat hepatoma and from normal liver and observed a definite lowering of cathepsin activity in the tumor extract, whereas dipeptidase and tripeptidase activities were equal in tumor and normal liver. Phosphatase from liver cancer induced by \( p \)-dimethylaminobenzene had an alkaline pH optimum, while the enzyme from normal liver had an acid pH optimum (42). Hepatoma and normal liver tissue also differ in their content of coenzymes, vitamins, etc. Thus the concentration of both ascorbic acid and glutathione is increased in a certain strain of transplantable rat hepatoma (22); this tumor is also rich in provitamin D, which is absent from \( p \)-dimethylaminobenzene hepatomas as well as from normal liver (35). No vitamin A could be detected in such hepatomas, or in four generations of transplants therefrom (23). Whereas the vitamin B\(_1\) content of these hepatomas is normal, that of cocarboxylase is decreased (43). They contain about one-fifth as much cytochrome c (18) and about one-seventh as much lactoflavin (33) as normal liver. The induction of liver tumors in rats by the dye is marked by a decrease of arginase and histidase in the nontumorous liver tissue (41).

It will be seen that most of the catalysts hitherto studied are components of the enzymatic equipment common to all living cells. In addition to these, hepatic tissue contains some highly specialized systems that are either specifically localized in the liver or are shared, usually in a much less active form, by one or a few other tissues. Any diminution or loss of these highly differentiated functions would be a chemical indication of the degree of dedifferentiation \(^1\) involved in the process of neoplasia.

We have therefore investigated, by the tissue slice method, the following reactions: urea synthesis, deamination of amino acids, oxidation of fatty acids, oxidation of uric acid, and synthesis of glycogen. The tumor material was the same as that employed in the investigation of respiration and glycolysis; i.e., rat hepatomas induced by feeding \( p \)-dimethylaminobenzene, and spontaneous hepatomas of old agouti mice.

**Sources of Material**

**Liver Tumors Produced by Feeding \( p \)-Dimethylaminobenzene**

**Feeding experiments.**—Two groups of 30 black-hooded rats each were fed rice to which had been added 20 ml. per kg. of a warmed 3 per cent olive oil solution of dimethyl yellow (B.D.H. analar). Carrot was added at the rate of one small slice per rat per day. Water was given ad libitum and the food was always present in the cages. The rice used was Egyptian, grown, of Japanese type, polished but not washed. Its vitamin B\(_1\) content was estimated (53) and found to be 0.23 \( \mu \)gm. per gm.

The first group of 30 rats (Nos. 1 to 30) received the rice without pretreatment. The second group (Nos. 31 to 60) were fed rice which had been extracted with diethyl ether by refluxing the unground rice (1 kg. batches) with two lots of the solvent (1 liter each). The ether extract was filtered off and the rice dried thoroughly in the air before use. On evaporation the ether yielded a clear yellow oil amounting to 0.2 to 0.3 per cent of the weight of rice taken; the properties of this oil were not investigated in view of the absence of any striking effect of the ether extraction on the incidence, type, or metabolism of the tumors or on the metabolism of the livers. The addition of rice-bran oil (ether-soluble substance) to the basic rice diet has been found by Kinoshita (33) and Sugiuara and Rhoads (59) to have a definite inhibiting effect upon the production of liver cancer in rats by the oral administration of \( p \)-dimethylaminobenzene. It is evident, therefore, that the polished rice used did not contain sufficient, or the right kind of, oil which was readily extractable by ether for this preliminary extraction to affect the incidence of tumors. It is noteworthy that in both the investigations referred to above large quantities of rice-bran oil (1 cc. per rat per day) were given.

**Tumor incidence.**—50 per cent of the animals in each group died before the 100th day of feeding; most of these showed no liver changes of interest for the present purpose and were not further examined. This high mortality agrees with that reported by Harada, Mizuta, and Maruya (29) and by Nakahara and his associates (45), and differs from the good survival observed by Sugiuara and Rhoads (59). There was an initial tendency to earlier death in the rats that received the ether-extracted rice. Cannibalism was not uncommon, and some material had to be discarded from this cause. Of a total of 22 rats which came to autopsy after having survived more than 140 days 19 had liver tumors, divided almost equally between the plain and ether-extracted rice groups (10 and 9 tumor-rats respectively). Of the ordinary rice group, 2 rats killed after 235 days had livers which were macroscopically normal as had also 1 rat of the ether-extracted rice group killed after 209 days. The other rats were killed at intervals for metabolic experiments between the 84th and 236th days.

**Nature of the liver changes.**—These have been so fully discussed (32, 40, 49, 21) that a brief reference.

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\(^1\) This term is used throughout without implying any specific type of mechanism by which the undifferentiated cell may arise.
to the main points will suffice. With one exception the tumors were examined histologically, and in many instances the surrounding liver tissue also. For these examinations we are indebted to Professor A. F. Bernard Shaw and Dr. J. G. Thomson. Definite macroscopic changes were first apparent in animals that died or were killed from the 84th day onwards. Cirrhosis was absent from the greater proportion of our specimens; it appeared to have been much less common than the descriptions of Orr (49) indicate for his material. Only two livers, in rats killed early in the experiment (84th and 107th days), showed typical nodular cirrhosis. Three other rats at the same period showed bile duct hypertrophy, not yet neoplastic, but no cirrhosis. All tumors present after the 140th day were histologically malignant.

The question of the origin of the tumors is at present open to discussion. The differing views of Orr (49) and the Japanese workers have been reviewed and given a detailed investigation by Edwards and White (21), without any final decision having been reached. Thus we merely report the results of routine histological examination without necessarily implying any significance as to the tissue of origin. Current views may be briefly summarized by saying that while the hepatoma is generally conceded to be derived from the parenchyma, there is no such unanimity with regard to the bile duct origin of the tumors that have histologically the appearance of cholangiomas. The question is important since the object of this investigation was to compare the metabolism of the tumors with that of their tissue of origin, and the problem will be mentioned again after the experimental results have been described.

Here our material will be considered only on a conventional histological basis. From this point of view there was the following distribution:

- Bile duct adenoma ........................................ 2 rats
- Bile duct adenoma with early carcinomatous change ... 6 rats
- Bile duct carcinoma with transition to liver cell carci-
  noma .................................................. 6 rats
- Liver cell carcinoma ..................................... 3 rats

Frequently the bile duct carcinoma in certain areas resembled liver cells, and in some instances apparent bile duct carcinoma was continuous with liver cell carcinoma. Sometimes, however, pure bile duct adenoma or carcinoma occurred, with no trace of liver cell carcinoma or transition thereto. In one such case special staining showed that many of the bile duct spaces and a number of the epithelial cells contained mucus, which may be an indication of their bile duct origin. We are indebted to Professor Shaw for this observation. The brief description applied to the material used for metabolic measurements, and included in the tables, is also based on these histological appearances.

**SPONTANEOUS HEPATOMAS OF THE MOUSE**

The tumors arose in old mice of Strong's CBA strain maintained by Mr. F. C. Pybus and Dr. E. W. Miller, to whom we are much indebted for this material. The occurrence and nature of these spontaneous hepatomas have been fully described by Strong and Smith (57, 58), Burns and Schenken (6), Gorer (24), and Pybus and Miller (51). The tumors, much more common in male than in female mice, were from 0.5 to 2.0 cm. in diameter, appearing usually as single masses projecting slightly above the surface of the organ and readily distinguished by their difference in color, and sometimes in texture, from the normal lobe. The surrounding liver was macroscopically normal. Microscopically the tumors are composed of liver cells (not bile duct), which differ but little in appearance from the normal. All the authors cited above agree on the generally benign tendency of the tumors. Thus Strong and Smith (57) state “The tumors seem to be of benign character, enlarging slowly by expansive growth.” Later the same authors (58) successfully inoculated one into related mice; all the grafts were surrounded by well formed capsules. The liver tumors observed by Burns and Schenken (6) in C3H mice “possessed some characteristics of both benign and malignant growths.” A blood vessel was invaded in one instance, yet the “remarkable differentiation of the tumor cells, the infrequency of mitoses, the absence of metastases, and the failure or extremely slow growth of the tumor transplants were suggestive, on the other hand, of benign tendencies.” Gorer (24) and Pybus and Miller (52) have observed pulmonary metastasis from one such tumor in each laboratory. In the latter metastasis, directly in the center of the lung, the architecture of the normal liver was preserved with astonishing completeness. We are specially indebted to Mr. Pybus and Miss Miller for directing our attention to this very interesting tumor, and to Dr. J. W. Orr for his comments; unfortunately there was no opportunity to undertake metabolic measurements with this specimen. The nature of these tumors may be summed up by saying that they are histologically almost identical with normal liver, but that they are capable of growth under favorable conditions when transplanted, and that very occasionally their metastasis has been observed. Their cause is unknown; leukocytic infiltration of the liver is sometimes present, and although a virus disease (infectious ectractemia) was present in some of Strong and Smith's (58) animals, the occurrence of virus and tumor could not be correlated.

**METHODS FOR METABOLIC STUDIES**

**Preparation of the tissue.—**The animals were killed by cervical dislocation when palpation, the lethargy of
the animals, or the duration of feeding in the p-dimethylaminationoazobenzene group, suggested that liver tumors might be present. The livers were excised and used immediately, a specimen being retained for histological examination. Razor slices of tumor and liver were prepared in Ringer's solution as usual, the greatest care being taken to avoid the contamination of liver by adjacent tumor, and vice versa. Macroscopically it was easy, as a rule, to distinguish tumor from liver, but occasionally it was difficult, in the p-dimethylaminationoazobenzene animals, to be certain that the organ was completely nontumorous, owing to the multiple nature of these growths. Sometimes they were too small or too diffuse for measurement, and these specimens were discarded. Sometimes only incomplete measurements could be made for a similar reason.

Respiration and glycolysis.—In the aerobic experiments 0.2 per cent of glucose was always present, but anaerobic glycolysis was frequently determined in both the presence and absence of glucose. For aerobic experiments in bicarbonate Ringer's solution (36) either the two-vessel method of Warburg (60) or, when the R.Q. was also to be measured, the method of Dickens and Simer (12) was used. The gas phase was oxygen with 5 per cent CO₂. In accordance with convention the anaerobic glycolysis (Q₉₀) in the Warburg two-vessel method is expressed on the assumption of an R.Q. of unity. This is obviously incorrect, but a simple calculation is all that is needed to transform the values of Q₉₀ for any chosen value of R.Q.

In the methods of Dickens and Simer (10, 12) we have now used for a long time equal wet weights of tissue, thus dispensing with the need for a separate determination of the bicarbonate content of the solutions. Also, by using for the R.Q. in bicarbonate-Ringer solution the convenient type of vessel described by Dickens and Greville (9), the amount of Ringer’s solution may be increased; normally we now use 2 cc. per vessel. Occasionally respiration and R.Q. in phosphate-Ringer solution (M/50 phosphate buffer, pH 7.4, in Krebs-Henseleit saline) were measured by the method of Dickens and Simer (10) in pure oxygen.

Anaerobic glycolysis was determined manometrically (60), in an atmosphere of nitrogen containing 5 per cent CO₂.

Glycogen was estimated by the method of Hynd and Rotter (31), with 1 gm. of fresh tissue, and by the Somogyi reagent (56) for estimating reducing power after hydrolysis.

Nucleic acid phosphorus was determined as described by Berenblum and his associates (1). The metabolic quotients (Q values) when expressed on the basis of nucleic acid phosphorus, in μgm., instead of dry weight of tissue, in mgm., are written PQ (1).

Special liver functions.—The tissue slices were shaken for 90 minutes with bicarbonate-Ringer solution to which the appropriate substrates had been added, in an atmosphere of oxygen containing 5 per cent CO₂, at 37.5° C. At the end of the incubation period the slices were removed, dried, and weighed, except in the experiments on carbohydrate synthesis. In these the results were based on the initial wet weight; in all other experiments they were based on the final dry weight.

After removal of the slices the solutions were analyzed by one of the procedures described below.

Urea synthesis.—As shown by Krebs and Henseleit (36), the synthesis of urea from added ammonium salts is optimal in the presence of traces of ornithine. The addition of a substrate, such as pyruvate or lactate, is favorable, too. Urea was determined by the method of Krebs and Henseleit (36), where the CO₂ liberated from urea by urease at pH 5 is measured manometrically. As our urease preparations (aqueous extracts of B.D.H. jack bean meal) showed a weak carboxylase activity, pyruvate somewhat interfered with the estimation of urea. dL-Lactate was therefore chosen as additional substrate; its final concentration was 1.74 × 10⁻³M. Ammonium chloride was present in a concentration of 8.7 × 10⁻³M and l(±)-ornithine dihydrochloride in a concentration of 8.5 × 10⁻⁴M.

Amino acid deamination.—l(±)-Alanine was used as the substrate in a final concentration of 9.1 × 10⁻⁴M. As the ammonia liberated from the amino acid is rapidly converted into urea by liver slices, it was estimated as urea.2 l(±)-Ornithine dihydrochloride in a concentration of 8.9 × 10⁻³M was added to insure the presence of sufficient catalyst for the maximal rate of urea synthesis. That under these conditions the rate of amino acid deamination is really the determining factor in normal liver is shown by the fact that the urea synthesis is much faster when ammonium ion is added than when alanine is added.

In some experiments the preformed urea was determined in both liver and tumor slices at the beginning of the experiment. Similarly, the formation of urea after 90 minutes' incubation without the addition of a urea precursor was measured. In both cases the amount of urea found was so small as to be almost within the limits of experimental error. The results obtained after 90 minutes' incubation with urea precursors therefore represent the true urea synthesis.

Fatty acid oxidation.—This was followed by measuring the formation of acetoacetic acid after incubation of the tissue slices with 4.4 × 10⁻⁴M sodium caprylate. After removal of the slices at the end of the incubation period the solutions were acidified with 1/10 volume of 50 per cent citric acid and acetoacetic acid.
acid was measured manometrically at 25° by addition of the aniline citrate reagent of Edson (19).

Uric acid oxidation.—Lithium urate was added to a concentration of 2.5 \times 10^{-3} M and its disappearance was measured colorimetrically. Trials with the manometric method of Edson and Krebs (20) were unsatisfactory. In this method, which is based on the conversion of uric acid into urea and glyoxylic acid, uric acid and allantoin have to be estimated separately. Uric acid is measured by the urea obtained after oxidation with MnO₂, followed by alkaline and finally by acid hydrolysis. Allantoin, on the other hand, is determined by the urea formed in an aliquot after alkaline and acid hydrolysis, but without previous treatment with MnO₂. We consistently found the allantoin too high, obviously because of an autoxidation of uric acid during the alkaline hydrolysis (20 minutes at 100°, pH about 10).

Uric acid was therefore estimated colorimetrically with the lithium arsenotungstate reagent of Newton (48). After removal of the slices, the solution (3.3 ml.) was deproteinized by the addition of 0.2 ml. of H₂SO₄ and 0.3 ml. of 10 per cent sodium tungstate. The measured filtrate was made up to 25 ml. and uric acid determined in a 5 ml. aliquot. Five ml. of arsenotungstate reagent (diluted 1:5) were added together with 15 ml. of 5 per cent sodium cyanide in 25 per cent urea and water to make up to 50 ml. A uric acid standard of 0.2 mgm. was used for comparison.

Synthesis of fermentable carbohydrate.—The tissue slices used for this experiment were divided into two roughly equal portions, drained on filter paper, and rapidly weighed on the torsion balance. They were then transferred to two manometer vessels containing 3 ml. of bicarbonate-Ringer solution and 0.3 ml. of 0.2 M pyruvate. One lot, which served for the estimation of the preformed carbohydrate, was immediately acidified by the addition of 0.5 ml. of 10 N H₂SO₄. The other vessel was shaken for 90 minutes at 37.5° C. before acidification in the same way. After acidification the vessels were put into a boiling water bath for 10 minutes. The contents of each vessel were then transferred to a mortar, thoroughly ground, and finally poured into a large test tube; 5 ml. of water + 3 ml. of H₂SO₄ were used, in several small portions, to rinse vessel and mortar. The test tubes were covered with glass bulbs and heated in a boiling water bath for 3 hours. After cooling, 1 ml. of 25 per cent NaOH and 1.3 ml. of 10 per cent sodium tungstate were added, the volume was measured, and the solution centrifuged. One ml. of acid cadmium sulfate solution and 0.4 ml. of 25 per cent NaOH were added to the measured supernatant solution, it was thoroughly mixed, and again centrifuged. The clear solution was tested with a drop of dilute NaOH solution and addition of NaOH was continued, if necessary, until precipitation was complete. When this was the case, the filtrate was neutralized to litmus.

The estimation of reducing power was carried out in 5 ml. aliquots, before and after fermentation, with the reagent of Somogyi (56). For fermentation 5 ml. of a 10 per cent suspension of well washed baker’s yeast were centrifuged and the solution to be fermented was added to the residue. After thorough mixing, the tube was left at room temperature for 15 minutes before it was again centrifuged and the residual reduction determined in the supernatant solution.

Units.—In order to conform with the notation of respiration and glycolysis all metabolic rates are expressed in Q values; i.e., the amount of metabolite produced or consumed, in gaseous form at N.P.T., expressed in microliters (1 millimol = 22.400 microliters) per hour, per mgm. of dry weight of tissue. The amount of urea and acetacetic acid is directly given by the quantity of CO₂ evolved, as one molecule of CO₂ is liberated from each molecule of these compounds. The rate of amino acid deamination is also expressed in terms of Qurea, but it is worth bearing in mind that each molecule of urea indicates the deamination of two molecules of alanine.

The results of the carbohydrate analyses are expressed in terms of glucose. The initial and final concentrations of carbohydrate are given as percentages of the initial tissue wet weight, but the metabolic changes are again converted into Q values, the final dry weight of tissue being here taken to equal 0.2 initial wet weight.

METABOLISM OF TUMORS INDUCED BY p-DIMETHYLAMINOAZOBENZENE

Ten histologically malignant tumors were obtained of size and purity sufficient for investigation. In three of these (Nos. 52, 53, 54) a full examination was possible, including respiration, glycolysis, and R.Q. of tumor and (except No. 53) liver, as well as urea formation, deamination, oxidation of fatty acid and uric acid, and synthesis of carbohydrate, in both tumor and liver. In the other examples the amount of available tissue limited the extent of the investigation to selected metabolic activities. Respiration and glycolysis of livers from rats 25, 26, 54, and 57 were compared with those of the tumors arising in these organs; stages preceding the formation of malignant tumors were studied in the livers of rats 15, 16, 24, 28, and 30; and respiration and glycolysis of the macroscopically tumor-free parts of the livers of rats 16, 25, 26, 54, and 57 were also measured. Metabolic activities other than respira-
tion and glycolysis were studied in both liver and tumor of rats 21, 52, 53, 54, 56, and 59.

The results of measurements of respiration and glycolysis are collected in Table II, while those of the other metabolic functions are given in Table III.

### Table II: Respiration, R.Q., and Glycolysis of Liver Tissue and Liver Tumors of Rats Fed p-Dimethylaminoazobenzene

<table>
<thead>
<tr>
<th>Rat No.* Method</th>
<th>Time of experiment, hours</th>
<th>$Q_{o2}$</th>
<th>$Q_{n2}$</th>
<th>$Q_{r2}$</th>
<th>Glyco-</th>
<th>Nucleo-</th>
<th>Remarks</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>gen,</td>
<td>protein</td>
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<tr>
<td></td>
<td></td>
<td>R.Q.</td>
<td>glucose</td>
<td>per cent</td>
<td></td>
<td>per cent</td>
<td></td>
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<tr>
<td>A. MACROSCOPICALLY NONTUMOR PART OF LIVERS</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>16 W ½</td>
<td>11.2</td>
<td>-0.8</td>
<td>+ 5.8</td>
<td>-</td>
<td>-</td>
<td>0.38</td>
<td>No cirrhosis</td>
</tr>
<tr>
<td>25 W 1½</td>
<td>9.4</td>
<td>+1.0</td>
<td>+ 5.3</td>
<td>-</td>
<td>+5.9</td>
<td>0.28</td>
<td>0.35</td>
</tr>
<tr>
<td>26 W 1½</td>
<td>8.4</td>
<td>+0.6</td>
<td>+ 6.3</td>
<td>-</td>
<td>+5.2</td>
<td>0.45</td>
<td>0.37</td>
</tr>
<tr>
<td>57 D &amp; S 2</td>
<td>11.0</td>
<td>+0.94</td>
<td>+ 3.5</td>
<td>0.92</td>
<td>+3.7</td>
<td>0.18</td>
<td>“ “ “ “</td>
</tr>
<tr>
<td>54 D &amp; S 2</td>
<td>11.0</td>
<td>+0.65</td>
<td>+ 5.2</td>
<td>0.86</td>
<td>+3.7</td>
<td>0.28</td>
<td>No cirrhosis</td>
</tr>
<tr>
<td>Average</td>
<td>10.2</td>
<td>+0.5</td>
<td>+ 5.2</td>
<td>0.89</td>
<td>+4.6</td>
<td>0.31</td>
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</table>

B. VARIOUS INTERMEDIATE STATES

<table>
<thead>
<tr>
<th>Rat No.* Method</th>
<th>Time of experiment, hours</th>
<th>$Q_{o2}$</th>
<th>$Q_{n2}$</th>
<th>$Q_{r2}$</th>
<th>Glyco-</th>
<th>Nucleo-</th>
<th>Remarks</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>R.Q.</td>
<td>glucose</td>
<td>per cent</td>
<td></td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 W 1</td>
<td>8.0</td>
<td>-3.2</td>
<td>+ 3.0</td>
<td>-</td>
<td>-</td>
<td>0.34</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td>24 D &amp; S 3</td>
<td>9.5</td>
<td>+2.2</td>
<td>+ 6.8</td>
<td>0.81</td>
<td>+4.0</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>30 D &amp; S 2</td>
<td>10.7</td>
<td>+3.0</td>
<td>+ 6.2</td>
<td>0.87</td>
<td>+3.3</td>
<td>0.37</td>
<td>“ “ “ “</td>
</tr>
<tr>
<td>28 W 2</td>
<td>10.5</td>
<td>+4.0</td>
<td>+ 6.2</td>
<td>-</td>
<td>+4.4</td>
<td>1.02</td>
<td>0.35</td>
</tr>
<tr>
<td>16 W ½</td>
<td>13.2</td>
<td>+2.6</td>
<td>+ 16.0</td>
<td>-</td>
<td>-</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>10.5</td>
<td>+1.0</td>
<td>+ 6.2</td>
<td>0.83</td>
<td>+3.8</td>
<td>0.34</td>
<td></td>
</tr>
</tbody>
</table>

C. LIVER TUMORS

<table>
<thead>
<tr>
<th>Rat No.* Method</th>
<th>Time of experiment, hours</th>
<th>$Q_{o2}$</th>
<th>$Q_{n2}$</th>
<th>$Q_{r2}$</th>
<th>Glyco-</th>
<th>Nucleo-</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R.Q.</td>
<td>glucose</td>
<td>per cent</td>
<td></td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 W 2</td>
<td>6.1</td>
<td>+5.9</td>
<td>+ 17.4</td>
<td>-</td>
<td>+1.8</td>
<td>0.11</td>
<td>0.46</td>
</tr>
<tr>
<td>25 W 1½</td>
<td>6.8</td>
<td>+0.6</td>
<td>+ 14.1</td>
<td>-</td>
<td>+1.9</td>
<td>0.16</td>
<td>0.46</td>
</tr>
<tr>
<td>53 D &amp; S 2</td>
<td>8.2</td>
<td>+5.3</td>
<td>-</td>
<td>-</td>
<td>0.89</td>
<td>Pure bile duct adenoma to carcinoma (mucus-containing)</td>
<td></td>
</tr>
<tr>
<td>57 D &amp; S 2</td>
<td>15.0</td>
<td>+7.8</td>
<td>+ 12.4</td>
<td>0.86</td>
<td>+1.5</td>
<td>0.51</td>
<td>Bile duct adenoma and liver cell carcinoma</td>
</tr>
<tr>
<td>54 D &amp; S 2</td>
<td>12.2</td>
<td>+6.6</td>
<td>+ 8.7</td>
<td>0.90</td>
<td>+4.0</td>
<td>0.38</td>
<td>“ “ “ “</td>
</tr>
<tr>
<td>52 D &amp; S 2</td>
<td>17.8</td>
<td>+5.9</td>
<td>+ 22.3</td>
<td>0.60</td>
<td>+2.1</td>
<td>0.65</td>
<td>Liver cell carcinoma</td>
</tr>
<tr>
<td>Average</td>
<td>12.0</td>
<td>+6.1</td>
<td>+12.5</td>
<td>0.79</td>
<td>+2.5</td>
<td>0.49</td>
<td></td>
</tr>
</tbody>
</table>

* Rats 1 to 30 received ordinary rice; rats 31 to 60 had ether-extracted rice.

† Since the nucleic acid metabolic quotients (PQ values) are based on the μgm. nucleic acid phosphorus, while the ordinary metabolic quotients (Q values) are based on the mgm. dry weight of tissue, the conversion of the above Q values to PQ values is given by:

$$PQ = \frac{Q}{\text{P}}$$

where $P$ is the percentage of nucleic acid phosphorus based on the dry weight of tissue (penultimate column).

### Respiration and Glycolysis

None of the livers examined can be regarded as strictly normal, in view of the prolonged administration of dye that preceded all experiments. Metabolically, however, there is little or no change in any of the "nontumor portions of liver" included in group A of Table II from the values typical of liver from normal rats on an adequate diet (13). The respiration and low aerobic acid production are certainly close to their expected magnitudes. The anaerobic glycolysis in normal liver is a somewhat uncertain quantity, owing to its dependence on the glycogen content (50) and previous treatment of the slices (55). The anaerobic glycolysis of adult liver is, however, predominantly a glycogenolysis (55), being little affected by the presence or absence of glucose in the medium. This is also true of the nontumor P-dimethylaminoazobenzene livers of Table II with the possible exception of No. 54, which apparently had some slight glucolytic as well as glycogenolytic power.

The states described as "intermediate" in Table II, group B, represent a collection based upon histological examination, which revealed at most a condition that might be described as an adenomatous growth of bile ducts without any histological indication of malig-
nancy. One example of cirrhosis, which as already mentioned, was rare in our series, has also been included. The duplicate estimations quoted for this specimen (No. 15) appear to show a considerable difference in metabolic activity in various regions of this liver; otherwise it was metabolically not unlike the normal organ. The other examples showed varying degrees of bile duct proliferation, and their metabolism (Nos. 24, 30, 28, and 16) shows no special change of respiration and R.Q. when compared with the normal. In all these examples, however, there is evidence of an appreciably increased aerobic acid formation, and in one instance (No. 16) of a greatly augmented anaerobic glycolysis compared with the nontumor portion of the same liver (Table II). In the remaining examples of this series the magnitude of $Q_{02}^N$ is not appreciably different from that of the nontumor parts, but it is probably significant that in each of the three cases studied (Nos. 24, 30, and 28) the anaerobic glycolysis is higher in the presence of glucose than in its absence. It seems that even in this early phase the cells of the liver have commenced to turn towards glucose in their anaerobic metabolism. This and the increased aerobic glycolysis are the only significant alterations at this stage.

The group (Table II, C) headed “liver tumors” includes only those examples which showed histological evidence of malignancy. On this basis they fall into two broad subgroups: (a) histologically bile duct carcinomas (Nos. 28, 25, 26, 53); (b) bile duct carcinomas containing also liver cell carcinoma (Nos. 57, 54, 52). The controversial nature of the interpretation of the histological picture in terms of the origins of the cells of these tumors has already been pointed out. All the tumors of this group were alike in the following respects. The aerobic glycolysis was in excess of that found for tissues of groups A and B; this excess was in every case quite clearly marked when compared with group A, and although not large for tumor 53 (with lowest $Q_{02}^N$ of group C) compared with intermediate state 28 (with highest $Q_{02}^N$ of group B), the general tendency to higher aerobic glycolysis in the malignant tumors was quite uniform. The increase in anaerobic glycolysis was usually well marked in the malignant tumors, and this difference becomes more striking when their relatively low glycolysis in the absence of glucose is taken into account. Tumors 25, 26, 53, 57, and 52 have turned almost exclusively to glycolysis as their anaerobic energy source. Curiously, tumor 54 (cf. incomplete measurements on tumor 28), which was an advanced tumor of considerable size and histologically undoubtably malignant, had an anaerobic metabolism almost identical with that of the early bile duct proliferation of intermediate state 30; comparison with the nontumor hepatic tissue of the same animal (No. 54, A and C) showed, however, some increase of anaerobic glycolysis and a 10-fold increase of aerobic glycolysis in the tumor. These determinations were run side by side and for the same duration; they are thus strictly comparable. The R.Q. of the liver tumors is of an order and range similar to that of normal liver, and resembles that of other tumors (11, 13, 5) in being below unity.

A comparison of the neoplasms according to their predominantly bile duct or liver structure shows that the only important difference in the two subgroups is a tendency to a lower respiration in the former (Nos. 28, 25, 26, 53) compared with the latter (Nos. 57, 54, 52). Tumors 57 and 52 had a particularly high $Q_{02}$, even though this was the average over 2 hours during which the respiration certainly fell somewhat; and the difference is not entirely explained by the increased cellularity of these tumors as indicated by their high content of nucleoprotein-phosphorus (cf. Table I, penultimate column). The histologically pure bile duct carcinoma of rat 53 had nucleoprotein-P intermediate in amount between liver cell tumors 57 and 52, but nevertheless had only about half their respiration. As far as the present data go, they indicate a high respiration of carcinoma cells of liver cell type. On the other hand, comparison of liver and tumor from Nos. 25 and 26 shows that the bile duct type of tumor has a lower respiration than that of the nontumorous liver tissue of the same animal, in spite of the higher nucleoprotein-P content of the tumor tissue.

Special liver functions.—Six tumors could be investigated (Table III). In 4 the whole series of functions could be studied, but in two the tissue available was sufficient only for the measurement of urea synthesis, deamination, and fatty acid oxidation. In three instances (Nos. 52, 53, 54) respiration, glycolysis, and R.Q. were determined on the same material. The tumors represented all histological types, ranging from structures resembling bile duct adenoma and carcinoma to pure liver cell carcinoma. There was, however, no clear cut difference in the metabolism of these histologically different types.

All the metabolic functions studied here were greatly reduced or had been lost entirely in the tumor tissue. But it is of interest that these functions disappear by no means in the same degree. The urea synthesis in particular usually persists at a measurable, though greatly reduced, rate, even after other reactions have ceased. This seems to favor a parenchymal cell origin for these tumors. They appear to be at different levels of dedifferentiation; thus tumor 52, which also has a relatively well conserved power of urea synthesis, still shows a considerable activity of deamination and fatty acid oxidation, though these are absent from some other tumors. No. 56 displays high
uricase activity, yet is unable to deaminate alanine or oxidize caprylic acid. In other cases there is not only no disappearance of uric acid but even an increase, as indicated by the positive quotient.

There is some variation, too, in the figures for the nontumorous livers. But though this was not neoplastic, it was not normal either, showing various degrees of degenerative, hypertrophic, or cirrhotic change. It must be remembered, furthermore, that certain activities, such as acetoacetic acid formation and glycogen synthesis, depend to some degree on the nutritional state of the liver. This is especially true for glycogen synthesis, which is most active if the liver contains little preformed carbohydrate (rat 59). The carbohydrate content of the tumors, though at a consistently very low level, is less variable than that of the surrounding liver, suggesting that it is less readily mobilized. Whereas all livers showed a considerable increase of fermentable carbohydrate after incubation with pyruvate, there was a decrease in two tumors. Two others exhibited a very small increase, almost within the limits of experimental error. Even if these increases be real, they show at least a greatly reduced capacity of synthesizing carbohydrate.

The objection may be raised that the small residue of the special hepatic functions which the tumor slices still possess is in reality due to contamination with surrounding liver tissue. This is very unlikely, as the tumor slices were prepared only from the central parts, all marginal portions being rejected. No islands of normal liver tissue were observed histologically inside the tumor. Furthermore, assuming an even distribution of this error, all values should be reduced to a similar degree, which is not the case.

### Table III: Analysis of Some Special Aspects of Liver Metabolism in p-Dimethylaminoazobenzene Tumors and in Adjacent Nontumorous Liver of the Rat

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Tissue</th>
<th>Urea formation Qnase. from NH₄⁺</th>
<th>Urea formation Qnase. from alanine</th>
<th>Acetoacetic acid formation Qnase. from Qnase.</th>
<th>Uric acid Qnase. from Qnase.</th>
<th>Total fermentable carbohydrate</th>
<th>Microscopic diagnosis of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Liver tumor</td>
<td>4.3</td>
<td>0.9</td>
<td>4.5</td>
<td></td>
<td>1.78</td>
<td>Liver cell carcinoma</td>
</tr>
<tr>
<td>52</td>
<td>&quot;</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
<td>1.78</td>
<td>0.14</td>
<td>Bile duct adenoma to carcinoma</td>
</tr>
<tr>
<td>53</td>
<td>&quot;</td>
<td>9.3</td>
<td>3.2</td>
<td>5.9</td>
<td>2.16</td>
<td>0.54</td>
<td>Bile duct adenoma to carcinoma</td>
</tr>
<tr>
<td>54</td>
<td>&quot;</td>
<td>3.0</td>
<td>0.9</td>
<td>1.2</td>
<td>+1.4</td>
<td>0.62</td>
<td>Bile duct adenoma to carcinoma</td>
</tr>
<tr>
<td>56</td>
<td>&quot;</td>
<td>9.6</td>
<td>3.2</td>
<td>5.9</td>
<td>0.62</td>
<td>0.54</td>
<td>+ liver cell carcinoma</td>
</tr>
<tr>
<td>59</td>
<td>&quot;</td>
<td>11.9</td>
<td>2.8</td>
<td>6.6</td>
<td>1.58</td>
<td>0.23</td>
<td>Bile duct carcinoma in places</td>
</tr>
<tr>
<td>Mean</td>
<td>Liver tumor</td>
<td>9.5</td>
<td>2.3</td>
<td>5.3</td>
<td>1.99</td>
<td>0.97</td>
<td>Liver cell carcinoma</td>
</tr>
</tbody>
</table>

The material was never sufficient for the measurement of respiration and glycolysis, and of the other metabolic activities studied, to be carried out on one and the same tumor. The similarities in the results obtained with different tumors in each series, however, make it extremely probable that these neoplasms have in general a fairly constant type of metabolism, and therefore the results of the two sets of investigations—of respiration and glycolysis, and of the other metabolic activities—may reasonably be compared one with another.

### Respiration and Glycolysis

The results for 7 tumors and the normal livers of their hosts are collected in Table IV. The most striking feature is the general similarity in the metabolism of liver and tumor, and this result contrasts strongly with the undoubted change of metabolism found in the highly malignant tumors of the rat (Table II) induced by p-dimethylaminoazobenzene.

Considering the results in detail, there are pronounced individual differences but none of these is systematically present, and it is highly probable that they are adventitious and to a large extent due to unavoidable chance selection of necrotic areas and other similar factors. It is known that in these livers and tumors pyknosis and other degenerative changes are frequent. This probably accounts for the fact that in some tumors the respiration is lower than that of the liver, and vice versa, and similar variations occur also in aerobic and anaerobic glycolysis.

In all examples except No. 4, the aerobic glycolysis...
of these tumors (and livers) is negligible. It is perhaps of importance that tumor 4, which showed considerable aerobic glycolysis in contrast to the liver tissue of the same animal, was permeated by polymorphonuclear leukocytes, especially lining the sinusoids, and showed greater variation than usual in the size and shape of the nuclei, of which many were hyperchromatic. Since that these neoplasms still perform all the functions of normal liver, as far as they were studied, at a rate of normal or only slightly impaired activity. Perhaps the very low level of carbohydrate in the normal liver of animal 12, compared with that of the hepatoma, again indicates that the carbohydrate of the hepatoma is less readily mobilized in response to peripheral demands.

Table IV: Respiration, Glycolysis, and R.Q. of Spontaneous Hepatomas of Mice, and of Livers of the Same Animals

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Method</th>
<th>Time of experiment, minutes</th>
<th>Hepatoma</th>
<th>Nucleo-protein-P, per cent</th>
<th>Normal lobe</th>
<th>Nucleo-protein-P, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>W</td>
<td>0-40</td>
<td>-0.1</td>
<td>2.1</td>
<td>-</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>0-30</td>
<td>1.1</td>
<td>1.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>D &amp; S</td>
<td>0-120</td>
<td>0.83</td>
<td>0.8</td>
<td>9.3</td>
<td>0.90</td>
</tr>
<tr>
<td>2</td>
<td>W</td>
<td>0-30</td>
<td>1.6</td>
<td>2.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>D &amp; S</td>
<td>0-120</td>
<td>0.8</td>
<td>0.9</td>
<td>8.5</td>
<td>0.88</td>
</tr>
<tr>
<td>3</td>
<td>W</td>
<td>0-30</td>
<td>3.9</td>
<td>4.9</td>
<td>9.2</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>D &amp; S</td>
<td>0-120</td>
<td>1.1</td>
<td>1.1</td>
<td>1.0</td>
<td>1.16</td>
</tr>
<tr>
<td>4</td>
<td>W</td>
<td>0-30</td>
<td>1.6</td>
<td>2.0</td>
<td>9.3</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>D &amp; S</td>
<td>0-120</td>
<td>1.3</td>
<td>1.3</td>
<td>1.2</td>
<td>1.22</td>
</tr>
<tr>
<td>5</td>
<td>W</td>
<td>0-60</td>
<td>1.3</td>
<td>2.3</td>
<td>1.3</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>D &amp; S</td>
<td>0-60</td>
<td>1.3</td>
<td>2.3</td>
<td>1.3</td>
<td>1.32</td>
</tr>
<tr>
<td>6</td>
<td>W</td>
<td>0-60</td>
<td>1.3</td>
<td>2.3</td>
<td>1.3</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>D &amp; S</td>
<td>0-60</td>
<td>1.3</td>
<td>2.3</td>
<td>1.3</td>
<td>1.32</td>
</tr>
<tr>
<td>7</td>
<td>W</td>
<td>0-60</td>
<td>1.3</td>
<td>2.3</td>
<td>1.3</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>D &amp; S</td>
<td>0-60</td>
<td>1.3</td>
<td>2.3</td>
<td>1.3</td>
<td>1.32</td>
</tr>
</tbody>
</table>

W = Two-vessel method of Warburg (60); D & S = phosphate-R.Q. method of Dickens and Simé (10).

Table V: Analysis of Some Special Aspects of Liver Metabolism in Spontaneous Mouse Hepatomas, Compared with Adjacent Nontumorous Liver

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Tissue</th>
<th>Urea formation from NH₃</th>
<th>Acetoacetic acid formation from caprylate</th>
<th>Uric acid oxidation</th>
<th>Total fermentable carbohydrate</th>
<th>Synthesis of carbohydrate</th>
<th>Initial, per cent</th>
<th>After 90 minutes, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Liver tumor</td>
<td>12.0</td>
<td>3.5</td>
<td>-6.3</td>
<td>5.7</td>
<td>6.4</td>
<td>0.07</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.8</td>
<td>-3.6</td>
<td></td>
<td></td>
<td></td>
<td>0.41</td>
<td>0.73</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>6.9</td>
<td>-3.6</td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
<td>0.66</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>7.3</td>
<td>3.9</td>
<td>-3.6</td>
<td>5.7</td>
<td>6.4</td>
<td>0.07</td>
<td>0.66</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>9.8</td>
<td>3.9</td>
<td>-3.6</td>
<td>5.7</td>
<td>6.4</td>
<td>0.07</td>
<td>0.66</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>7.9</td>
<td>3.9</td>
<td>-3.6</td>
<td>5.7</td>
<td>6.4</td>
<td>0.07</td>
<td>0.66</td>
</tr>
</tbody>
</table>

leukocytes have been reported (39) to possess high aerobic glycolysis in Ringer's solution, it is possible that part of the metabolic activity of this specimen may be accounted for by the infiltration.

In general, however, the respiration, glycolysis, and R.Q. of these tumors are of the same type as those of normal mouse liver.

Special liver functions.—Unfortunately the number of tumors available for the investigation of special liver functions was small, and these usually did not yield enough slices for the whole series of analysis. But such experiments as there are (Table V) quite clearly show

METABOLISM OF NORMAL SKIN, LEUKOPLAKIA, AND CARCINOMA, OF THE VULVA

This human material was selected because the whole series of changes from normal, through the intermediate leukoplakia which is widely regarded as precancerous, and finally to squamous cell carcinoma may be followed. We are indebted to the Gynaecological Department of the Royal Victoria Infirmary, and especially to Dr. S. Way, for this material, which was used fresh immediately after excision in the form of razor slices. The subcutaneous fatty layer was carefully separated from the cuts and the latter was cut into
to measure the R.Q. of leukoplakia (e.g., triplicates, 0.94, 0.98, 1.06), and these measurements have also been omitted from Table VI, which summarizes the results. The experimental conditions were the same as those previously described, and the Ringer’s solution contained 0.2 per cent glucose.

Four samples of skin, 3 of leukoplakia, and 3 of carcinoma were examined. In two cases skin and carcinoma from the same patient were used; in the others glycolysis are about the same in these two normal tissues. The PQ values may be compared with those of Berenblum, Chain, and Healey (2) for normal isolated epithelium of the rabbit’s ear, viz. 
PQO₂ = 0.90; PQH₂O + 0.45; PQH₂ + 1.3; (R.Q. 0.7). In view of the different material the correspondence is so good that here at least there seems little need of separating the nearly pure epithelial layer and using the elaborate ultra-micromethods of Berenblum and his colleagues,

### Table VI: Metabolism of Normal Skin, Leukoplakia, and Carcinoma, of the Vulva

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Method</th>
<th>Time of experiment, minutes</th>
<th>( Q_0 )</th>
<th>( Q_0^{0.8} )</th>
<th>( Q_0^{0.2} )</th>
<th>R.Q.</th>
<th>(-PQO_2)</th>
<th>( PQO_2^{0.8} )</th>
<th>( PQO_2^{0.2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>W</td>
<td>0–120</td>
<td>0.74</td>
<td>+0.20</td>
<td>+0.89</td>
<td>—</td>
<td>0.62</td>
<td>+0.17</td>
<td>+0.71</td>
</tr>
<tr>
<td>4</td>
<td>D &amp; S (a)</td>
<td>0–120</td>
<td>0.64</td>
<td>1st hour +1.05</td>
<td>2nd hour +0.79</td>
<td>—</td>
<td>0.53</td>
<td>+0.87</td>
<td>+0.66</td>
</tr>
<tr>
<td>6</td>
<td>D &amp; S (b)</td>
<td>0–120</td>
<td>1.08</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.08</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>W</td>
<td>0–60</td>
<td>0.02</td>
<td>+0.33</td>
<td>+0.72</td>
<td>—</td>
<td>1.5</td>
<td>+0.54</td>
<td>+1.2</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>0.86</td>
<td>0.27</td>
<td>+1.15</td>
<td>—</td>
<td>0.93</td>
<td>+0.36</td>
<td>+0.89</td>
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</table>

#### NORMAL SKIN OF VULVA

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Method</th>
<th>Time of experiment, minutes</th>
<th>( Q_0 )</th>
<th>( Q_0^{0.8} )</th>
<th>( Q_0^{0.2} )</th>
<th>R.Q.</th>
<th>(-PQO_2)</th>
<th>( PQO_2^{0.8} )</th>
<th>( PQO_2^{0.2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>W</td>
<td>0–60</td>
<td>2.14</td>
<td>+2.60</td>
<td>+8.3</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.78</td>
<td>+2.39</td>
<td>+7.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.27</td>
<td>+0.31</td>
<td>+1.15</td>
<td>—</td>
<td>0.85</td>
<td>+0.21</td>
<td>+0.77</td>
</tr>
<tr>
<td>5</td>
<td>W</td>
<td>0–90</td>
<td>0.69</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>D &amp; S (a)</td>
<td>0–105</td>
<td>0.87</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.58</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>D &amp; S (a)</td>
<td>0–105</td>
<td>0.93</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.62</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>W</td>
<td>0–60</td>
<td>1.36</td>
<td>+1.29</td>
<td>+3.43</td>
<td>—</td>
<td>1.07</td>
<td>+1.02</td>
<td>+1.91</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>1.29</td>
<td>+1.65</td>
<td>+5.00</td>
<td>—</td>
<td>0.72</td>
<td>+0.62</td>
<td>+1.34</td>
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</table>

#### SQUAMOUS CARCINOMA OF VULVA

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Method</th>
<th>Time of experiment, minutes</th>
<th>( Q_0 )</th>
<th>( Q_0^{0.8} )</th>
<th>( Q_0^{0.2} )</th>
<th>R.Q.</th>
<th>(-PQO_2)</th>
<th>( PQO_2^{0.8} )</th>
<th>( PQO_2^{0.2} )</th>
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<tbody>
<tr>
<td>2</td>
<td>W</td>
<td>0–60</td>
<td>2.23</td>
<td>+13.3</td>
<td>+21.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.79</td>
<td>+13.8</td>
<td>+24.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>D &amp; S (b)</td>
<td>0–135</td>
<td>2.55</td>
<td>+11.0</td>
<td>+23.2</td>
<td>0.95</td>
<td>0.94</td>
<td>+4.05</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.44</td>
<td>+10.4</td>
<td>+23.2</td>
<td>0.95</td>
<td>0.90</td>
<td>+3.84</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.39</td>
<td>+13.6</td>
<td>—</td>
<td>0.88</td>
<td>0.88</td>
<td>+5.02</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>D &amp; S (a)</td>
<td>0–120</td>
<td>3.52</td>
<td>1st hour +14.8</td>
<td>2nd hour +11.4</td>
<td>0.95</td>
<td>1.30</td>
<td>—</td>
<td>+5.48</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3.90</td>
<td>—</td>
<td>—</td>
<td>0.88</td>
<td>1.44</td>
<td>—</td>
<td>+4.22</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>2.69</td>
<td>+12.4</td>
<td>+20.7</td>
<td>0.92</td>
<td>1.09</td>
<td>+4.31</td>
<td>+6.09</td>
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</tbody>
</table>

W = Two-vessel method of Warburg (60); D & S (a) = phosphate-R.Q. method of Dickens and Simére (16); D & S (b) = bicarbonate-R.Q. method of Dickens and Simére (17). The PQ values are the Q values calculated on the basis of mgm. of nucleic acid phosphorus, instead of on a dry weight basis (cf. Berenblum, Chain, and Healey, 1). Nucleic acid-P in the carcinoma of case 3 was assumed to be the same as carcinoma of case 4; all other nucleic acid-P values were determined.
since the much more readily applicable Warburg tech-
nic gives substantially the same results in this case.

The Q values of leukoplakia all tend to be increased
as compared with skin, particularly the anaerobic, and
to a less extent the aerobic, glycolysis. The PQ values
of aerobic and anaerobic glycolysis are also increased.
However, these changes are slight compared with the
entirely different type of metabolism shown by the
carcinoma. Here the Q values of respiration are only
about double those of the leukoplakia, but the anae-
robic and aerobic glycolyses have both risen to the high
values typical of the majority of malignant tumors.

Naturally, since the relative magnitudes of the Q
values are quite different in the tumors, compared with
their relative values in skin and leukoplakia, the PQ
values show the same kind of alteration. Considering
the absolute increases, when these are based on the
nucleic acid phosphorus as a supposed indication of the
metabolically active tissue substance only, it is seen that
the increase in aerobic glycolysis (0.4 to 4.3) and ana-
erobic glycolysis (0.9 to 6.1) is very great in the car-
cinoma compared with normal skin. Thus the met-
abolism of the tumor is not only qualitatively different in
type from that of the skin, but the activity of its
Cellular elements appears to be much greater than that
of the cellular material in skin.

These findings are in complete contrast to those of
Berenblum and his associates (2) with Shope papil-
loma and rabbit epithelium. These authors' values for
the papilloma are in good general agreement with those
obtained by us for human leukoplakia, and the ques-
tion arises whether the tumors used by them were
really malignant. In view of our results, it is very
desirable that this point should be investigated with
histologically controlled material, and the changes fol-
lowed right through to the stage of malignancy.

**DISCUSSION**

Histologically the two types of liver tumors studied
in this investigation stand at opposite poles. Whereas
the highly differentiated spontaneous mouse hepatomas
closely imitate the structure of normal liver, the
growths induced by p-dimethylaminoazobenzene are,
at least in their final stages, definitely malignant and
are composed of cells of a much more primitive ana-
plastic type. Whether these cells are originally derived
from bile duct epithelium or from parenchymal cells is
not of great importance from our point of view, since
they might be expected to revert ultimately to the same
primitive type, in view of their common embryological
origin.

Our studies reveal the same picture in the field of
metabolism; the spontaneous mouse hepatomas are
almost indistinguishable from normal liver, not only
with respect to respiration and glycolysis, but also in
their capacity to perform some very specialized and
organ-specific functions. The p-dimethylaminoazo-
benzene tumors, on the other hand, differ radically
from normal liver in the following ways: They have
acquired the power to ferment glucose both aerobically
and anaerobically, a property common to almost all
malignant growths. Though the extent of their respira-
tion is not much changed, they retain only a small
residue of, or have completely lost, the specialized
oxidative functions particular to liver cells; viz., forma-
tion of urea from /-amino acids, oxidation of fatty acids
and of uric acid, and also other synthetic functions
which depend on respiration: synthesis of carbohydrate
and ura. This result shows how superficial an impres-
sion a mere recording of the net oxygen uptake gives
of the respiratory activity of any tissue. Nor does the
respiratory quotient in these cases where the R.Q. of
the original tissue is low give any indication of the
deep seated metabolic changes which have occurred.

The p-dimethylaminoazobenzene tumors thus pre-
sent a metabolism which is highly dedifferentiated
compared with that of normal liver. The appearance
of fermentative glucose breakdown which persists in
oxygen is, in our view, merely another aspect of the
same transformation, or reversion to a more primitive
type of metabolism, such as is shown by fibroblasts and
epithelial cells when these are grown in tissue cultures
(61, 37, 38).

Two objections may be raised against this view:
(a) Aerobic glycolysis is lacking in the whole embryo,
except in very early stages and then only after removal
from the embryonic membranes (47), and it is also
absent from embryonic liver or kidney (55) although
embryonic liver still retains some ability to attack
acetone anaerobically (54). (b) Normal adult tissues
exist which have a high aerobic glycolysis, although
they are highly differentiated (see Introduction).

These objections, however, are not necessarily in-
compatible with the general conception advanced, since
(a) even the very young embryo already possesses some
degree of differentiation; (b) the aerobic glycolysis of
certain normal adult tissues, of which we have made a
special study (14, 15, 16), may perhaps be regarded as
an adaptation to the inadequately oxygenated environ-
ment in which these cells are situated within the body,
as we have previously suggested. Thus the reversion
to fermentation as a primitive source of energy in
these normal tissues is not necessarily bound up with
an all-round dedifferentiation. In the malignant tumor
cell, on the other hand, the primary change is associated
with an all-round dedifferentiation including, amongst
other things, a reversion to the primitive type of fer-
mentative metabolism.4

---

4 It should be noted that some of the "histologically typically
malignant" mouse tumors of Murphy and Hawkins (44) had
no aerobic glycolysis, so it cannot be asserted that aerobic gly-
colysis is always a necessary consequence of this reversion. Direct
evidence of the state of tissue oxygenation in vivo, in relation
to tissue metabolism, is urgently needed in all these cases.
The extent of this reversion of metabolism in tumor cells varies, in the examples studied in this paper, in proportion to their deviation in morphological character and in invasive growth from the properties of normal tissue; that is to say, it varies by and large with the extent to which these cells have taken on the character of malignancy. Considered from this point of view, the nonlycizing spontaneous mouse hepatomas and leukoplakias of the vulva are examples of conditions which still retain a high degree of differentiation; their growth is orderly and the blood supply may therefore be adequate. In these cases it is only when the normal differentiation of the cell is lost, i.e., at a stage which corresponds in general with the onset of malignancy, that the metabolism is definitely changed, and we consider it probable that malignant transformation always implies a change of metabolism, even if the over-all values of respiration, glycolysis, and respiratory quotient should remain unaltered. In such cases only a detailed study of the enzyme systems which make up the metabolic equipment of the cell will reveal the changes that have occurred.

In previous discussions much controversy has centered around the question of a specific tumor metabolism. There is no known single feature of tumor metabolism that is not possessed by one or other type of normal cells; on the contrary, the tumor cell has lost most of the specialized functions of normal cells: hence there is little reason to expect any one metabolic property to be exclusively characteristic of the tumor cell. In fact, no such specific property has been found. It is to be expected that any biological difference between any two cells will ultimately be reflected in their chemistry and metabolism. The closer the biological relationship, however, the more difficult will be the detection of these subtle chemical differences.

SUMMARY

The metabolism of tumors of the liver and skin has been studied in relation to that of their tissue of origin.

Two types of liver tumors have been used: (a) malignant liver tumors induced by feeding p-dimethylaminoazobenzene to rats; (b) highly differentiated spontaneous hepatomas arising in agouti mice.

The skin tumors studied were carcinomas of the human vulva, and these were compared with the intermediate leukoplakia and normal skin of the vulva.

The respiration, glycolysis, and respiratory quotient of malignant liver tumors were similar to those found generally in tumor metabolism; viz., high anaerobic and moderately high aerobic glycolysis combined with an R.Q. below unity. In confirmation of Orr and Stickland (50), it was found that in these tumors the glycogenolysis typical of normal adult liver is replaced by glucose breakdown. Precancerous states of the liver induced by p-dimethylaminoazobenzene, showed only a slight increase of aerobic glycolysis, and in some cases a slight anaerobic glycolysis also. The benign spontaneous mouse tumors had respiration, glycolysis, and R.Q. substantially the same as that of the adjacent liver tissue.

In addition to the measurements just described, the following highly specialized functions were studied in both types of hepatic tumor, and were compared with the surrounding nontumorous liver tissue: formation of urea from ammonia, formation of urea from \( \text{f}(+)\)-alanine, formation of acetoacetic acid from caprylic acid, oxidation of uric acid, synthesis of fermentable carbohydrate from pyruvic acid.

These highly differentiated functions were entirely, or almost entirely, lost in the malignant tumors induced by p-dimethylaminoazobenzene, though urea synthesis usually persisted at a measurable but greatly reduced rate even after the other reactions were lacking. On the other hand, the spontaneous mouse hepatomas still retained almost intact all the foregoing functions of the normal liver cell.

The respiration and glycolysis of carcinoma of the vulva were typical of those of malignant growths in general, both aerobic and anaerobic glycolysis being high. On the other hand, the leukoplakia showed only a slight increase of both the latter reactions as compared with the normal skin of the vulva.

The interpretation of none of these results is affected by the use of the nucleic acid phosphorus content of the tissue, instead of its dry weight, as a basis for the calculation of the metabolic values.

The loss of special liver functions by the p-dimethylaminoazobenzene tumors is regarded as a reversion to a more primitive type of metabolism. It is suggested that the appearance of glycolysis, aerobically and anaerobically, in this as well as in other tumors, is a metabolic expression of the same primary process of dedifferentiation.

REFERENCES


22. FUJITAWA, T., NAKAHARA, W., and KIMI, S. Comparison of Chemical Composition between Hepatoma and Normal Liver Tissues. VI. Ascorbic Acid and Glutathione. Gann, 32:107-115. 1938.


34. KISHI, S., FUJIWARA, T., and NAKAHARA, W. Comparison of Chemical Composition between Hepatoma and Normal Liver Tissues. VII. Cathespin, Dipeptidase and Tripeptidase. Gann, 33:469-476. 1938.


52. Pybus, F. C., and Miller, E. W. Personal communication.
It is imperative (1) to continue the study of the biology of the tumor cell, particularly human, in the fields of karyology, metabolism of substances, and physico-chemical properties, (2) to extend work on the elucidation of the possibility of isolating the factor of malignancy from mammalian cells.

On the most important question of precancerous states are being conducted numerous investigations. Side by side with extensive clinical observations on the understanding of precancer, the question was enlightened by morphologic, experimental, biologic, and biochemical points of view. On this were received certain new data on the general and local changes in the organism during the precancer period.

The question requires further detailed study in close collaboration with the clinic.

On the question of resistance and disposition to cancer the following results were reached: (1) It was established that the organism possesses resistance to malignant neoplasms; the stimulation of this characteristic of the organism constitutes one of the most important problems in the war against cancer. (2) It was established that active mesenchyme plays an essential role in the protection of the organism to blastomatous growth (a view introduced by Acad. A. A. Bogomoletz 14 years ago). (3) The stimulation of active mesenchyme by doses of specific cytotoxic serum restores the carcinolytic property of serum of patients with cancer, which points toward the possibility of achieving a beneficial effect on the mesenchyme of patients with cancer. (4) Data were presented allowing the suggestion that the increase in immunity to malignant tumors is associated with an increase in oxidizing processes and lowering of glycolytic processes, and, conversely, lowered resistance is associated with depressed tissue respiration and increased glycolysis.

Essential are (1) further studies on the role of active mesenchyme in the pathogenesis of malignant tumors, (2) further studies on the action of cytotoxic antigens in the tumor with the objective of developing methods of prophylaxis of recurrences and metastases and general treatment of cancer, (3) further studies in the question of specific immunity to malignant tumors, on the antigenic properties of the cancer cell, and possibly anti-tumor vaccination, (4) further studies of the bio-chemical nature of the immunizing substance to malignant tumors.

New experimental results were presented showing the role of the nerve component in the distribution of metastases. Further wide study of this question is essential, as well as of the whole problem of the nervous system in the origin and course of tumors.

Reports were presented on the subject of cachexia in cancer, from clinical as well as experimental standpoints. Special attention was devoted to the importance of full-value protein and vitamin diet in patients with cancer. Further work on the pathogenesis of cancer cachexia through investigations in metabolism is essential.

For the guarantee of the possibility of extending the investigations noted above, the convention considers essential: (1) Organization of laboratories of organic synthesis for the production of carcinogenic and antineoplastic chemical compounds. (2) Organization of laboratories for biologic investigation, on animals, of the possible carcinogenic action of compounds suspected of possessing such properties and having importance in industry or which may be widely used by the general population. (3) The initiation of studies, in special oncolgic institutes, on the problem of biologic and chemical methods of cancer therapy and the study of methods of biologic diagnosis. (4) It is considered desirable to obtain from the United States or France a series of homozygous strains of mice and other laboratory animals and to assure their maintenance in several central laboratories. (5) To procure strains of tumors that are important for experiments and not available in the U.S.S.R., (6) To initiate the problem of producing new strains of tumors (of dogs, monkeys, and other animals). (7) To develop methods of producing tumors by chemical products in new types of animals.

M. B. Shein


The "Dictionary of Bio-Chemistry," as stated in its preface, was designed for readers of biochemical literature. The book is a cross between an alphabetical glossary and something resembling a condensed encyclopedia. However, whether a book of this type is a mere glossary or an encyclopedia, its value depends entirely upon its degree of accuracy in defining biochemical terms and compounds. This book contains misleading information and so many inaccuracies and poor definitions that, despite some good but brief articles, it cannot be recommended for readers of biochemical literature. To mention only a few of its shortcomings and errors, coenzyme I is said to be a mononucleotide and not differentiated from coenzyme II; lysozyme, which is a protein, is stated to contain no nitrogen; and urea is said to react in vitro with glycine to form glycocyanine. Also, the configuration of some of the natural amino acids is sometimes said to be levo- and sometimes dextro-. Anyone wishing to understand the recent controversy over Kögel's theory on the occurrence of 4-amino acids in tumors would find this book a hindrance rather than an aid.

David Shemin

Correction

The authors of The Metabolism of Normal and Tumor Tissue (3:73-87, 1943) request that the following correction be made. It is made thus tardily because their first letter respecting it was lost in transit.

In Tables III and V the second column, which now reads:

<table>
<thead>
<tr>
<th>Liver tumor</th>
<th>Liver tumor</th>
<th>Liver tumor</th>
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</thead>
<tbody>
<tr>
<td>etc.</td>
<td>etc.</td>
<td>etc.</td>
</tr>
</tbody>
</table>

should read as it was in their manuscript:

<table>
<thead>
<tr>
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<th>Liver</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Tumor</td>
<td>Tumor</td>
</tr>
<tr>
<td>etc.</td>
<td>etc.</td>
<td>etc.</td>
</tr>
</tbody>
</table>
The Metabolism of Normal and Tumor Tissue. XX. A Comparison of the Metabolism of Tumors of Liver and Skin with That of the Tissue of Origin

F. Dickens and H. Weil-Malherbe


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