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Serum and Liver Transaminase Activities in Experimental Virus Heptatitis in Mice

An increase (20- to 40-fold) in the aspartic-ketoglutaric and alanine-ketoglutaric transaminase activities of the serum in human epidemic virus hepatitis was found by us (*1, 2*). Later, aspartic-ketoglutaric transaminase activity in epidemic hepatitis was investigated by Wróblewski and LaDue (*3*), and comparable results were obtained.

No such increase has been found in any other type of icteric or anicteric liver diseases that were investigated by Wróblewski and LaDue (*3*) and by us (*1, 2*), with the exception of myocardial (precocious) infarction (*4*). Furthermore, these authors found an increase in the aspartic-ketoglutaric transaminase activity of serum both in cases of carbon tetrachloride poisoning in human beings and in cases of experimental carbon tetrachloride poisoning in mice (*3*).

In epidemic hepatitis, the increase in alanine-ketoglutaric transaminase activity is more consistent than the increase in aspartic-ketoglutaric activity. Consequently, the ratio of aspartic-ketoglutaric transaminase activity to alanine-ketoglutaric transaminase activity (As-K/Al-K) falls from the normal (mean) value of 1.3 to 0.64 in acute epidemic hepatitis (*1, 2*). Such a pattern is retained by the serum of a patient during his convalescence, when the absolute values of the enzymatic activities of the patient's serum are no longer diagnostically significant. We have suggested that such enzymatic determinations could be of use as a diagnostic test for human epidemic hepatitis (*1*). Our later experiments (63 cases of epidemic hepatitis) fully confirm our first results (*5*).

Questions have arisen with regard to the following: (i) whether the increase of the enzymatic activities is really related to the destruction of parenchymal liver cells as suggested by us and others (*1–5*); (ii) whether such enzymatic variations are common features of all human (epidemic, yellow fever, infectious mononucleosis, and so on) and animal virus hepatitides; (iii) whether enzymatic variations that are observed in serums are associated with variations in the same activities in liver tissue; (iv) whether the possible enzymatic variations in liver tissue are analogous or opposite to those in serums from the same subjects.

The present results (Table 1) concern two enzymatic activities of the livers and serums of mice infected with 1000 LD_{50} of hepatitis virus (MHV-3 Craig, of Gledhill and Andrewes, *6*) and killed on the fourth day of the disease (*7*). Determinations were made according to Tonhazy, White, and Umbreit (*8*).

Determinations were made of enzymatic activity in homogenates of liver tissue from each of 15 infected mice and each of 15 normal mice and in five pools of serums from normal mice and five from infected mice. Each pool contained a mixture of serums from three animals. The results obtained led us to the following conclusions:

1) An increase is shown in the investigated transaminase activities in the serums of the animals experimentally infected with MHV-strain virus. This increase is comparable to that observed in human beings with epidemic hepatitis. Furthermore, the afore-mentioned decrease in the value of the As-K/Al-K ratio is found in the serum both in cases of human hepatitis and cases of experimental hepatitis in mice.

2) The variation in either of the transaminase activities in the liver (both decrease) of an animal is opposite to the variation in the analogous transaminase activity in the serum of the same animal but roughly proportional to the latter in magnitude. The decrease in the alanine-ketoglutaric transaminase activity in livers and the increase in this activity in serums are more pronounced than the changes in activities of aspartic-ketoglutaric transaminase in serums and livers.

These conclusions support the hypothesis that the enzymatic variations observed are not limited to human virus hepatitis (*9*). Furthermore, the necrosis of hepatic cells seems to play an important role in the pathogenesis of the phenomenon (passage from the liver to the blood of enzymatic metabolites).

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Table 1. Transaminase activities at 37°C of livers and serums of mice. Aspartic-ketoglutaric transaminase activity is measured in micromoles of oxalacetate formed, and alanine-ketoglutaric transaminase activity is measured in micromoles of pyruvate formed.

Item	Normal mice	Infected mice	± %	Student's *t*
*Liver**				
Oxalacetate	115.1	86.6	− 25	4.31†
Pyruvate	111.6	58.8	− 48	5.93†
As-K/Al-K	1.03	1.46	+ 41	3.70†
Serums‡				
Oxalacetate	2.98	31.7	+ 999	3.98§
Pyruvate	0.95	37.7	+3867	4.44§
As-K/Al-K	3.04	0.84	− 75	4.42§

* Averages of 15, 100 mg of tissue for 10 minutes. † t significant > 2.763 ($P = 0.01$), $G = 28$. ‡ Averages of five pools, each from three of the same mice, 1 ml for 15 minutes. § t significant > 3.355 ($P = 0.01$), $G = 8$.

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Consistent Biochemical Pattern in Malignant Tumors

In a previous study (*1*), a variety of normal and malignant tissues were subjected to cell fractionation by differential centrifugation. In all of the malignant tissues studied, the apportionment of protein among the various cell fractions showed a remarkably consistent pattern in which the nuclear and final supernatant-fluid fractions contained most of the protein and in which the mitochondrial and microsome fractions contained relatively little. The original series included 15 fractionations of nine malignant animal tumors, but only two human tumors.

Recently it has become possible to extend the observations to four more malignant human tumors (*2*). These included a lymph node affected by Hodgkin's disease, an inguinal lymph node containing an epidermoid carcinoma metastatic from the penis, a fibrosarcoma superficial to the posterior costal margin, and a cer-

vical lymph node containing an epidermoid carcinoma of undetermined primary site. All of the specimens were chilled immediately after removal at surgery and were maintained below 3°C throughout the fractionation procedure (*1*).

It was found that the human tumors (Table 1) varied considerably in concentration of protein nitrogen and ribose nucleic acid (RNA) in each cell fraction when the concentrations were expressed as the amount per unit of desoxyribose nucleic acid (DNA) in an equivalent amount of the whole tissue (*3*). On the other hand, when the quantity of protein nitrogen, or RNA, in each cell fraction was expressed as a percentage of the total amount present in the tissue (Table 2), it became evident that all of these malignant human tumors showed a consistent pattern in the distribution of protein among the cell fractions. In all of these tumors, the nuclear and final supernatant-fluid fractions each contained approximately 40 percent of the protein, whereas the mitochondrial and microsome fractions each contained only about 10 percent of the protein. This pattern is the same as that found previously in the animal tumors (summarized in Table 2), which included three rat hepatomas induced by different carcinogens, three transplantable rat tumors (Jensen sarcoma, Flexner-Jobling carcinoma, and Walker-256 carcinosarcoma), and three transplantable mouse tumors (a mammary carcinoma, an adrenal carcinoma, and a lymphosarcoma).

Three distinct patterns were found in the distribution of protein among the cell fractions of the normal tissues studied previously (Table 2). The first pattern, that of the thymus and possibly the adrenal, was similar to that of the tumors, with the bulk of the protein in the nuclear and supernatant-fluid fractions and relatively little in the mitochondria and microsome fractions. The second pattern, which is characteristic of liver and kidney, differed from that of the thymus and tumors by having a relatively large portion of the protein in the mitochondrial fraction. The pancreas and submaxillary gland provided the third pattern, which differed from that of the thymus and tumors by the presence of a relatively large portion of the protein in the microsome fraction. It seems probable that the distribution of protein found in the thymus and tumors represents the complement of cell components normally present in relatively unspecialized tissues, and that the large quantity of mitochondria in liver and kidney, as well as the large quantity of microsomes in pancreas and submaxillary gland, reflects the development of additional equipment required for the special functions of these tissues. These interpretations are consistent with the recognized importance of the mitochondria in the production of energy for the cell, in carbohydrate and lipid metabolism, and in esterification reactions (*4*). They are also consistent with the recent work that suggests a dominant role for the microsomes in the synthesis of protein (*5*).

Table 1. Concentration of protein-nitrogen and RNA in cell fractions from malignant human tumors.*

Tumor	Protein nitrogen†				Ribose nucleic acid†			
	Nuc	Mt	Micro	Super	Nuc	Mt	Micro	Super
Hypernephroma‡	2.76	0.49	0.74	1.89	0.62	0.12	0.21	0.30
Fibroscarcoma No. 1‡	1.75	0.32	0.46	1.31	0.50	0.14	0.28	0.37
Hodgkin's disease	0.50	0.17	0.18	0.49	0.04	0.03	0.11	0.09
Epidermoid carcinoma No. 1	0.88	0.21	0.21	0.69	0.25	0.11	0.20	0.23
Fibrosarcoma No. 2	2.26	0.76	0.27	2.10	§	§	§	§
Epidermoid carcinoma No. 2	0.89	0.07	0.16	0.64	0.17	0.02	0.14	0.26

* Expressed as milligrams of protein-nitrogen or RNA in a given tissue fraction per milligram of DNA in an equivalent amount of whole tissue (*2*).
† Nuc, nuclear fraction; M, mitochondria; Micro, microsomes, Super, supernatant fluid.
‡ From previous study (*1*).
§ Ribose nucleic acid samples accidentally lost during processing.

Table 2. Percentage distribution of protein and RNA among cell fractions of various normal and malignant tissues.*

Tissue	Protein nitrogen†				Ribose nucleic acid†			
	Nuc	Mt	Micro	Super	Nuc	Mt	Micro	Super
Human tumors								
Hypernephroma	47	8	13	32	50	10	17	24
Fibrosarcoma No. 1	46	8	12	34	39	11	22	29
Hodgkin's disease	37	13	13	37	15	11	41	33
Epidermoid carcinoma No. 1	44	11	11	35	32	14	25	29
Fibrosarcoma No. 2	42	14	5	39				
Epidermoid carcinoma No. 2	51	4	9	36	29	4	24	44
Animal tumors‡	38 ± 5	8 ± 3	13 ± 2	40 ± 3	32 ± 9	10 ± 3	29 ± 5	30 ± 5
Normal tissues§								
Thymus, rat	58, 60	3, 3	8, 7	32, 32	0, 15	3, 3	37, 30	61, 51
Adrenal, human	20	13	17	51	16	9	37	37
Liver, rat	14, 17	34, 30	19, 17	32, 36	10, 12	28, 27	47, 41	15, 20
Liver, mouse	21	32	11	37	11	39	29	21
Kidney, rat	16, 17	26, 27	17, 16	40, 40	14, 15	13, 17	32, 31	40, 37
Pancreas, rat	14, 16	8, 9	32, 34	46, 40	4, 6	2, 2	51, 55	42, 37
Salivary gland, rat	11, 29	8, 8	29, 27	52, 36	9, 23	3, 3	54, 48	35, 25

* The quantity of protein nitrogen or RNA in each cell fraction is expressed as a percentage of the total protein nitrogen or RNA present in the tissue.
† Nuc, nuclear fraction; Mt, mitochondria; Micro, microsome; Super, supernatant fluid.
‡ Average values, ± mean deviation, obtained in 15 fractionations of nine different animal tumors, calculated from individual values previously reported (*1*).
§ Where two values are given, they were obtained from two different experiments.

Although the distribution of protein within the tumor cells was similar to that in the thymus, the tumor cells differed from these, and from all the other cells studied so far, in the high proportion of RNA in the nuclear fraction (Table 2).

Many previous studies have shown that, for a wide variety of biochemical properties, malignant tumors show a narrower range of values than do normal tissues (*6*), but the data presented here appear to offer the first demonstration of a consistent biochemical pattern in malignant tumors among at least four variables—namely, the proportion of cell protein to be found within each of the four cell fractions. This pattern was found without exception in the cells of a wide variety of malignant tumors, including primary and metastatic human tumors, induced and transplanted animal tumors, carcinomas as well as sarcomas, in three different animal species. In all of the tumors studied, the nuclear and final supernatant-fluid fractions each contained approximately 40 percent of the protein, and the mitochondria and microsome fractions each contained about 10 percent of the protein. In most of the tumors studied, the nuclear fraction contained 30 percent or more of the RNA. This was considerably more than the amount found in the nuclear fraction of any of the normal tissues studied.

Thus, while malignant tumors show manifold differences in their histologic

appearance (differences which provide the basis for clinical diagnosis), and although they show subtle differences in many biochemical properties, the consistent pattern demonstrated here in the distribution of protein among the various parts of the cell indicates that, within their cells, all of the tumors studied possess the same basic architecture.

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Antiserotonins in Hypertension and the Antimetabolite Approach to Chemotherapy

In 1944, as a result of experiments with antimetabolites of vitamins and purines, Woolley (*1*) suggested that useful pharmacological agents could be made which would control specific noninfectious diseases. This could be accomplished by use of the antimetabolite idea coupled with knowledge of vitamin or hormone deficiencies. Thus, a disease arising from an excess of one of the hormones present in an animal might logically be controlled by production of a structural analog of this hormone that would act antagonistically to it. This idea was an extension of that of Woods (*2*) and Fildes (*3*) regarding the mechanism of control of bacterial infections by sulfanilamide.

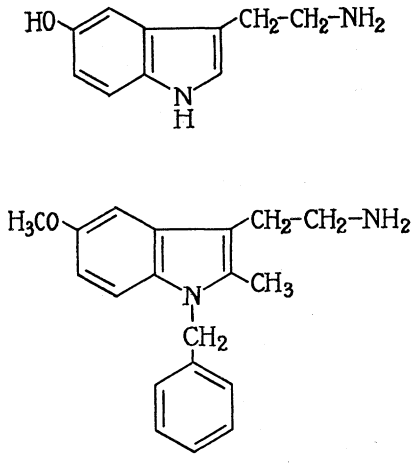

Fig. 1. Structures of serotonin (top) and 1-benzyl-2,5,dimethyl-serotonin or BAS.

The prospect that was thus raised of a rational chemotherapy of both noninfectious and infectious disorders through the use of antimetabolites has, in the intervening years, been viewed with considerable skepticism by many investigators. These have reminded us that penicillin and other wondrous agents have not been discovered in this fashion but rather have been found empirically—for example, by screening programs. Because this opposing view has enjoyed such acceptance, we would like to record the following facts as an interesting case in which the antimetabolite approach has led to the development of a new series of pharmacological agents capable of checking a common human disease.

In 1952 we (*4*) suggested that essential hypertension might arise from an excess of serotonin. We also saw that if this were so, the disease might be controlled with a suitable antimetabolite of serotonin. The first antiserotonins were thus envisioned and produced. We have pursued this idea and have made a member of this series of antiserotonins which is apparently suitable.

The compound in question is 1-benzyl-2,5-dimethyl serotonin, a benzyl analog of serotonin, or BAS. The structures of BAS and of serotonin are shown in Fig. 1. This antimetabolite was synthesized in the manner described previously (*5, 6*) and was found to be highly effective by the oral route in protecting dogs from the pressor action of serotonin (*6, 7*). However, it was not merely a hypotensive agent, because, in normal dogs and in one normotensive human being, it did not lower blood pressure significantly. What it did do was to protect these animals against the pressor effects of injected serotonin.

As a result of these findings, the aid of Robert Wilkins of the Massachusetts Memorial Hospital was sought in order that a clinical trial of this antimetabolite could be made. Preliminary findings reported by Hollander, Michelson, and Wilkins (*8*) have shown that BAS will bring about reduction in blood pressure of patients with hypertension. Only small doses were needed, and these did not seem to cause harmful side-effects.

Several problems arose during the discovery of BAS, and some are worthy of comment here. (i) It was realized at the outset that a drug, to be useful in this disease, must be orally effective. (ii) The drug should also have a low inhibition index, and it should be an irreversible antagonist of serotonin (*9*). This was desirable so that the serotonin that is constantly being produced in the body would not readily nullify the effects of the drug. (iii). The compound must not affect the central nervous system so as to cause mental disturbances. This was a very real problem, because early in the investigation of antiserotonins we had found (*10*) that some of them do disturb the mind. It was therefore necessary to design one that would not elicit this kind of effect.

The antiserotonin, BAS, was so designed that it met these requirements. For example, the benzyl group was introduced to help with points i and ii. The validity of this can be seen from the fact that the corresponding debenzylated compound was shown to lack the high activity by the oral route and to lack some of the irreversible character (*7*). The primary aliphatic amino group was retained in the molecule to deal with point iii. Several compounds with the amino group either attached at other points or otherwise modified caused profound behavioral changes in animals (*10*).

The findings of Wilkins and his collaborators (*8*) show that a suitably constructed antimetabolite of serotonin such as BAS can bring about a reduction in the blood pressure of many hypertensive patients and can do this without causing obvious harmful side-effects. Whether or not such a compound will be a useful drug remains to be seen. It is quite probable that further alteration of the structure of serotonin to give other members of this series of antimetabolites will be needed in order to achieve the best agent. Nevertheless, present facts indicate that the original hypotheses are worthy of attention. These hypotheses are (i) that a new series of drugs may be discovered with the antimetabolite idea, and (ii) that serotonin may be causally related to essential hypertension.

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