Tissue Distribution of Boron Compounds in Relation to Neutron-Capture Therapy of Cancer.* (21010)

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The promising direct application of the atomic pile to the treatment of patients with malignant brain tumors, investigated by Farr, Sweet, *et al.* at Brookhaven National Laboratory during the last two years, has focussed attention on a number of problems relating to the biological behavior of boron compounds. This mode of cancer therapy is based on the fact that certain nuclides, notably boron-10, disintegrate on capturing a neutron with the release of high energy heavy particles.† By virtue of their charge, their internal origin and other factors, these particles appear to have a therapeutic effectiveness some 10 to 20 times that of equivalent energy as x-rays. Moreover, their relatively large mass permits them even at these high energies to travel an average of only 10-15 μ in tissue. This means that the lethal disruptive effect from the disintegration of that atom is confined to a zone only twice the diameter of a red blood cell. The beauty of this concept, first proposed on theoretical grounds by Kruger(1), and later experimentally by Zahl, Cooper and Dunning (2), resides in the fact that in boron-neutron treatment biologically effective radiation is induced only at the site of interaction of two components which are in themselves relatively innocuous, whereas in the usual administration of radioactive isotopes for therapeutic purposes, radiation is delivered to whatever parts of the body the isotopes may reach, often concentrating in such undesired places as normal liver and bone marrow. A neutron beam on the other hand can be directed to any desired portion of the head or body. Its practical usefulness, however, requires a sufficient concentration of boron in the cancer tissue, a favorable ratio between this concentration and that of the surrounding normal tissues and, finally, a high enough flux of *thermal* neutrons at the cancer site. Conger and Giles(3) presented the first quantitative evaluation of the biological effects of this neutron-boron reaction in plants, and their lucid exposition led Sweet(4) to propose that neutron-capture therapy might be applied with unique advantage to the treatment of brain tumors. He, with Javid(5) demonstrated, after the intravenous injection of borax, useful concentration ratios between malignant tumors and normal brain of some 3 or 4 to 1. This paved the way for the actual clinical trial of neutron-capture therapy with boron-10, which has thus far been carried out in 10 patients for a total of 21 irradiations at the Brookhaven National Laboratory reactor(6).

These early studies have brought up the following fundamental questions: 1) Is tissue concentration of borate, and hence effective radiation during neutron bombardment, proportional to the injected dose? 2) How is borate partitioned between the extracellular and intracellular tissue compartments? (The latter favors greater cell nucleus disruption by the secondary particles.) 3) Does the distribution of borate suggest that neutron-capture therapy may be applicable to neoplasms other than brain tumors?

Methods. In other communications it was demonstrated that pure-strain mice bearing transplantable malignant brain tumors provide a useful means of assaying distribution and biological behavior of various radioactive and stable isotopic compounds, and that such information can be carried over with reasonable confidence to predict the behavior of the

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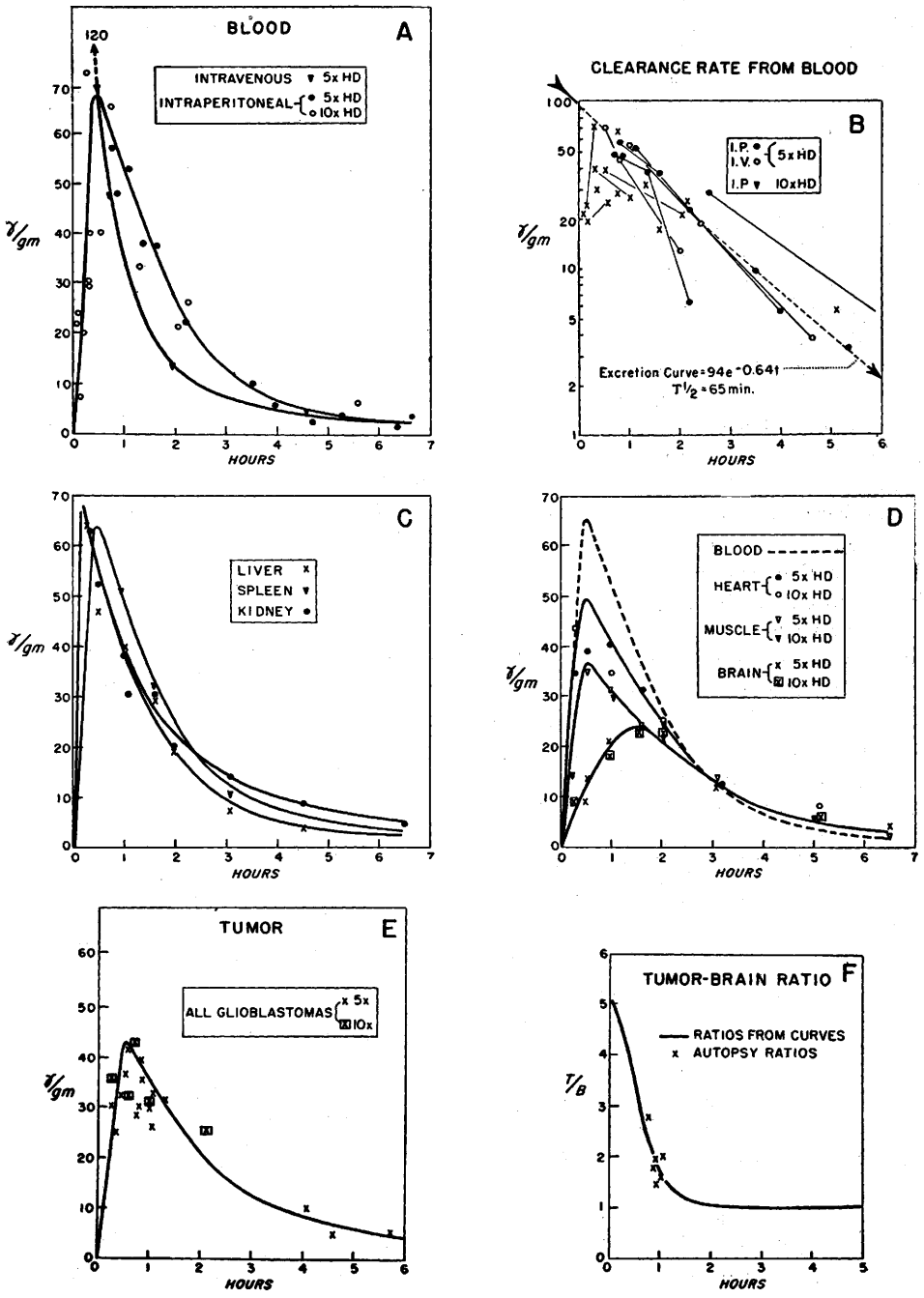
† Expressed as a nuclear equation:
$_5B^{10} + {}_0n^1 = [{}_5B^{11}] = {}_3Li^7 + {}_2\alpha^{4++} + 2.79$ Mev
In this interaction, boron has a capture cross section of 3900 barns. The unstable B^{11} disintegrates in less than a microsecond into an ionized lithium nucleus and an alpha particle, and the 2.79 Mev of free energy is divided between them as kinetic energy approximately in the inverse ratio of their masses.

DISTRIBUTION OF BORAX IN MICE*

INTRAPERITONEAL INJ: 5x HUMAN DOSE$^{\Psi}$ = 36 γ BORON (0.02 ml) PER GRAM BODY WT.



*DATA EXPRESSED AS MICROGRAMS (γ) BORON PER GRAM WET TISSUE
ΨSEVERAL MICE RECEIVED 10x HD; THEIR TISSUE LEVELS PLOTTED TO ½ SPACE

FIG. 1.

same agent in human brain and brain tumors (7,8). This mouse-assay technic has been applied to the present studies of boron compounds, and details of the experimental method may be found in these two reports. The mouse brain tumors used in our experiments were induced by implantation of 20-methylcholanthrene according to the method of Seligman and Shear(9) in the laboratory of Dr. Harry Zimmerman, and they have passed through more than 50 generations of subcutaneous transplantations in mice of the same strain. We have used 2 types classified by Zimmerman as glioblastoma and astrocytoma. Boron has been studied as borate in the form of borax, and since there is no radioisotope of boron suitable for tracer work, the data have been obtained by chemical analysis, using the method of Ellis, Zook and Baudisch (10). The borate curves for man, presented for comparison, were drawn from a replotting of data by Sweet and Javid(5). Their studies were all done during operations on patients with highly malignant brain tumors, when it is possible to obtain small samples of brain and tumor at intervals after injection from the area of the brain being resected, without compromise to the patient.

Results. Body distribution and excretion. The distribution characteristics of borate administered intraperitoneally in mice are shown in Fig. 1. Absorption and distribution to the tissues are very rapid, and by 30 minutes all organs, except brain, have reached a peak. The various organs form a family of similar curves such that the faster the rise in concentration, the higher the peak and the earlier its occurrence. Thereafter the level in blood and tissues falls off rapidly; and since there is apparently no tissue reservoir soaking up large amounts of borate at this time, this sharp decline can reasonably be attributed to excretion. After roughly three hours all the tissues, including the brain, appear to be in equilibrium and losing borate at about the same rate, suggesting that their levels are now being controlled largely by the excretory mechanism. This is well illustrated by the tumor-brain ratio curve (Fig. 1F): from its initial value of 5 or 6 to 1 it falls sharply to around 2:1 by one hour, and remains about 1:1 from two

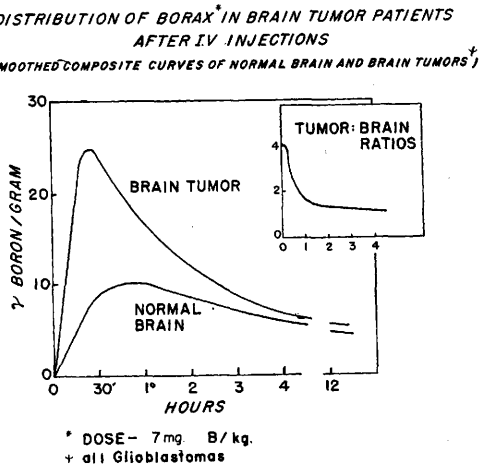


FIG. 2.

hours to the end of the curve. Having observed the rapidity and importance of excretion, we made an indirect calculation of its rate. Fig. 1B is a semilog plot of all our blood concentration data in mice, with lines connecting serial samples from the same mouse. After the peak is reached, the decline in blood levels (followed also by the tissue levels) is very nearly linear, indicating that excretion proceeds roughly as a simple exponential function; and from the average of the various slopes, the excretion half-time is determined to be 65 minutes.‡ One mouse, sacrificed two hours after injection, was found to have a full bladder, a fortuitous situation in view of the usually irresponsible excretory habits of these creatures. This enabled us to determine that the concentration of borate in the urine was 20 times the 2 hour plasma concentration and 5 to 10 times the mean plasma level during the 2-hour period. From this we judge that borate is cleared by the kidney as a "low-threshold substance" in a class with urea and sulphate, tubular reabsorption of which is primarily by passive diffusion.

Comparison with human data. In Fig. 2 the distribution of borax between tumor and brain in man is shown for comparison. A remarkable similarity in behavior may be noted: the peaks occur at about the same

‡ It will be seen later on more refined analysis that the disappearance curve has two exponential components.

TABLE I. Tissue Levels of Borate* vs. Dosage in Mice.

Mouse #	Time	Dose†	Route	Blood		Heart	Muscle	Brain	Liver	Spleen	Kidney	Dose ratio‡	Avg tissue ratio‡
				Plasma	RBC								
V-14	31′	.25X	S.C.	1.8	1.7	1.6	—	.3	1.6	—	—	.05	.03
15	35′	.6 X	”	5.0	4.0	6.3	—	2.6	5.7	—	6.4	.12	.16
17	30′	2.0 X	”	14.2	15.0	15.4	—	3.2	13.0	—	13.0	.40	.33
S.C.§	*32′*	*5.0 X*	*I.P.*	*68*		*45*	*42*	*14*	—	—	—	—	—
V- 3	70′	.25X	I.P.	.7	1.0	1.0	—	.6	.8	—	1.5	.05	.03
20	65′	.6 X	”	2.6	2.9	3.4	—	2.2	3.0	—	3.6	.12	.095
6	65′	2.0 X	”	8.1	7.0	7.8	—	5.4	7.9	—	9.8	.40	.25
S.C.	*65′*	*5.0 X*	”	*47*		*38*	*32*	*21*	*37*	—	*37*	—	—
II-5	62′	10.0 X	”	54		70	61	36.2	—	—	—	2.0	1.8
V- 9	2h	.6 X	I.P.	2.4	1.6	1.9	—	—	2.4	—	2.4	.12	.09
S.C.	*2h*	*5.0 X*	”	*26*		*35*	*22*	*21.5*	*21*	*24*	*24*	—	—
II-4	2h	10.0 X	”	42.4		50	50.2	44	—	—	—	2.0	2.0
V- 2	4.5h	.6 X	I.P.	.54	.88	.51	—	.56	.48	—	1.0	.12	.12
S.C.	*4.5h*	*5.0 X*	”	*4.5*		*6.0*	*6.5*	*5.5*	*3.5*	—	*7.0*	—	—
S.C.	*6.7h*	*5.0 X*	*I.P.*	*2.8*		*2.0*	*2.6*	*4.2*	*2.0*	*3.0*	*4.5*	—	—
I-42	6.6h	15.0 X	”	4.5		5.0	5.5	5.1	4.5	4.5	8.2	3.0	1.8
I-43	6.8h	20.0 X	”	4.4		3.1	3.3	4.5	3.5	2.6	—	4.0	1.4

* Conc. expressed as γ boron/g wet tissue.

† Dosage factors are in terms of human dose of 70 μg borax (7.1 γ boron)/g body wt at operation, used in studies by Sweet and Javid.

‡ Dose-ratios and tissue-ratios are all compared to 5X human dose used in obtaining standard distribution curves in mice; the figures for brain have not been included for the first 30 min. in obtaining average tissue ratios.

§ S.C. = Standard curve for mice. (Whole blood data used.)

TABLE II. Distribution* of Borate in Normal and Nephrectomized† Mice.

	Mouse	Dose‡	Route	Time	Plasma	RBC	Heart	Brain	Liver	Kidney
A:	V-14	.25X	S.C.	31′	1.8	1.7	1.6	.3	1.6	N
	15	.6 X	"	35′	5.0	4.0	6.3	2.6	5.7	6.4
	16	.6 X	"	34′	4.6	5.2	5.5	2.4	6.0	N
	17	2.0 X	"	30′	14.2	15.0	15.4	3.2	13.0	13.0
	18	2.0 X	"	30′	15.3	14.4	13.4	5.5	14.4	N
B:	V- 8	.25X	I.P.	2h	1.4	1.4	.8	.5	1.0	N
	10	.6 X	"	2h	4.6	3.8	—	3.4	4.6	N
	12	2.0 X	"	2h	12.8	12.7	10.3	9.8	13.0	N
C:	V-16	.6 X	S.C.	34′	4.6	5.2	5.5	2.4	6.0	N
	10	.6 X	I.P.	2h	4.6	3.8	—	3.4	4.6	N
	1	.6 X	"	4h	5.3	4.7	4.9	3.7	5.0	N

* Conc. expressed as γ boron/g wet tissue.
† Nephrectomized mice indicated by N under "Kidney."
‡ Dosage factors refer to human dose of 70 μg borax (7.1 γ boron)/g body wt.

time, the tumor-brain ratio curves are nearly identical, and the rate of excretion is roughly parallel. There is one important discrepancy, however, which requires explanation. Whereas the curves for mice are based on a dose 5 times that used in man, their tissue levels are only 2 to 2½ times those seen for brain and tumor in man. From question (1) above the reader will readily understand the importance of this discrepancy to the calculation of tissue levels and radiation dosage during neutron-capture therapy.

Tissue levels vs. dosage. To test this relationship further, the ratio of tissue levels was studied in a series of mice given doses varying from 0.25 to 20 times the standard dose used by Sweet and Javid in patients at operation. The data, presented in Table I, indicate that tissue levels are in fact roughly proportional to dosage over the range from 0.25 X to 10 X for at least the first two hours, and that with 0.6 X and 5 X doses they are proportional up to 4.5 hours. (Only in the 15 X and 20 X doses at 6.7 hours is there serious disagreement—to be discussed later.) The 10 to 20% differences seen between the dose-ratios and the average tissue-ratios are within the experimental error of the method. Since in boron-neutron treatment, irradiation is carried out during the first hour, these data indicate that the concentration in brain and tumor may be calculated with confidence from the data of Sweet and Javid multiplied by the appropriate dosage factor. To test the effects of excretion on the proportionality of tissue levels and dosage, we did bilateral nephrectomies on a series of mice and carried out a parallel distribution study in them and a group of their normal confreres. The nephrectomized mice behaved with normal vigor and curiosity during the 24 hours they were studied and seemed to differ from the normal controls only in their abstinence from food and water. In order that they might not be jeopardized, they were given doses closer to the safe levels used in patients at operation rather than the 5-fold dose used in the mice of our control experiment (Fig. 1).

In Table IIA, tissue levels in normal and nephrectomized mice measured 30 minutes after injection of three dosage levels are compared. At this early time, both groups are seen to have about the same tissue levels for the same dose, and in both groups tissue levels and dosage are directly proportional. That this rough proportionality persists even when excretion stops is seen in Table IIB where 3 dosage levels are compared at 2 hours in nephrectomized mice.

In Table IIC, which shows tissue levels at varying times, but with the same dosage in nephrectomized mice, it is seen that equilibrium has been reached in heart, liver and red cells by 30 minutes, whereas brain continues to rise during the entire four hours. Mouse No. 16 was injected subcutaneously. Since this did not impair its tissue levels, which if anything were a little higher, it is concluded that borate is rapidly absorbed by all parenteral routes. The above studies indicate that up to 30 minutes, rates of absorption and distribution are the crucial factors determining

TABLE III. Distribution* of Borate at Equilibrium in Nephrectomized Mice.

Mouse	Dose†	Route	Time	Plasma	RBC	Brain	Heart	Liver
14	.25X	S.C.	31′	10	9.4	1.6	9.0	9.0
16	.6 X	"	34′	10.6	11.8	5.4	—	13.6
18	2.0 X	"	30′	10.0	9.4	3.6	8.7	9.4
10	.6 X	I.P.	2h	10.5	8.6	7.7	—	10.5
1	.6 X	"	4h	12.0	10.7	8.4	11.2	11.4
		Avg		10.6	10.0		9.7	10.8

* Conc. expressed as γ boron/g. Tissue levels for each mouse uniformly normalized by appropriate factor to a dosage of 10 γ/g.

† Dosage expressed in terms of standard human dose of 70 μg borax (7.1 γ boron)/g body wt.

the peak concentrations. They soon yield, however, to the dominant effects of excretion, which is apparent from the much lower levels seen in normal mice 30 minutes later (c.f. the 65-minute group of Table I). Since the discrepancy between mice and man resides fundamentally in the peak concentrations occurring at about 30 minutes, it seems likely from the above that the different routes of administration constitute the responsible factor. Since the tumor concentration in both species falls to one-half the peak values in roughly an hour (and this fall has been shown to be due to excretion) it may be concluded that excretion proceeds at a similar pace in the two species.

Compartmental distribution of borate. When a substance is injected into the body, there are 3 basic distribution patterns it may follow: 1) It may behave primarily as an extracellular constituent, like sodium, and distribute uniformly throughout the plasma, interstitial fluid and lymph—a volume representing some 20% of the body weight. 2) It may be distributed throughout the body water, both extracellular and intracellular, in essentially uniform concentration, in which case the diluting volume is about 65 to 70% of body weight. 3) It may reside primarily within the cells of the body, as is the case with potassium, and be concentrated many fold compared with the plasma level. In this case, the *apparent* diluting volume calculated on the basis of the plasma concentration may even exceed the body weight.

We have applied these principles to an analysis of the distribution data in nephrectomized mice in which we felt more confident that a true distribution equilibrium was reached. In Table III the data for the nephrectomized mice have been normalized to a standard dose of 10 γ boron per gram of body weight. From the averages of the equilibrium values, one notes that the concentration of boron in most of the organs, plasma and red cells is practically identical, and that the diluting volume is 100% of the body weight, suggesting the intracellular pattern of distribution (see No. 3, previous paragraph). Since plasma is about 92% water by weight, and the tissues about 75%, a uniform and exclusive distribution in body water would require that the tissue concentrations be about 82% that of the plasma. Actually they turn out to be 90-100%, and we have sought a particular reservoir for the additional 10-20% in specific abdominal organs, bone, and fat. None of these accounts for the excess, which must then represent an intracellular accumulation above that in general body water.

In the light of these conclusions it is reasonable to expect that the turnover rate of borate in its bound intracellular form will be different from the exchange rate of the freely diffusible ion. A more detailed graphic analysis of the blood data presented in Fig. 1B, supplemented with data extending out to 24 hours, shows in fact that its clearance is more accurately represented by 2 exponentials having the following formula:§ Excretion $= 88\, e^{-\frac{.693}{60}t} + 6\, e^{-\frac{.693}{330}t}$ where t is in minutes. From this equation we may deduce that if injection and mixing were instantaneous, the initial level would be 94 γ/g (the sum of the

§ In this formulation the denominator of each exponential is its half-time.

coefficients), representing a dilution volume some 35 to 40% of the body weight. Evidently, at the peak, borate is distributed beyond the extracellular space but incompletely in the intracellular space before excretion begins to play a substantial role. This equation indicates further that about 94% (88/94) of the boron in the body is excreted with a half-time of 60 minutes (compared with the earlier estimate of 65 minutes for the total clearance rate), and the remaining 6% with a half-time of 5.5 hours. Thus the faster of these represents the renal excretion rate of the freely diffusible borate, and the slower one would appear to be the turnover rate of the bound fraction.

These two phases of borate distribution also bear an important relationship to the proportionality between dosage and tissue levels. We have seen in the dosage study that during the early hours after injection (*e.g.*, during the predominantly aqueous phase of distribution) dosage and tissue levels are directly proportional over a wide range. However, with heavier doses this correlation broke down at 6.7 hours, since then the tissues all contained roughly 5 γ/g of boron regardless of whether the dose was 5 X or 20 X. Apparently, when the aqueous phase has been largely depleted by excretion, the role of the tissue-bound borate becomes relatively greater and affects the proportionality adversely.

Factors determining tumor-tissue ratios. We have seen that borate is distributed rapidly to tumor and the various organs *except brain,* and that high, useful concentrations and tumor-brain ratios occur during the first hour after injection, when rates of absorption and distribution are the controlling factors. Both concentrations and ratios fall precipitously after the first hour, however, due to the controlling effect of renal excretion. Although within the first hours after injection borate is distributed uniformly throughout much of the body, with the passage of time the normal brain retains a greater and greater percentage of that which remains. Thus the concentration in the brain in our nephrectomized mice was still rising at 4 hours; in one of our rabbits at 24 hours the borate in the brain had risen to 75% of that in the other tissues; whereas in Pfeiffer's dogs(11) at 7 days the brain contained a higher concentration than any other organ. This unusual predilection for nervous tissue, with its accompanying toxic effects, limits the frequency with which boron-neutron treatments may be given. Happily this relative affinity does not become manifest at once so that during the initial period of rapid exchange and high concentrations, when most of the boron is in the aqueous phase, the brain concentration is relatively low and the tumor-brain ratio of a useful magnitude. In other parts of the body where borate diffuses freely and there is little specific affinity among the organs or tumor, the concentration ratios are essentially unity. Unfortunately this state of affairs offers little immediate promise for the use of boron-neutron treatment for neoplasms other than those of the central nervous system.

Summary and conclusions. 1) Recent application of the atomic pile to the neutron-capture therapy of human brain tumors has focussed attention on the biological behavior of boron compounds. 2) Studies reported in mice and man indicate that borax and boric acid are rapidly absorbed and distributed throughout the body. Uptake by the various organs and tumor describes a family of similar curves such that the faster the rise in concentration, the higher and earlier is the peak. All tissues studied, except brain, reach a peak by 30 minutes. Borate is rapidly excreted by the kidneys as a "low-threshold substance" in a class with urea and sulphate, and clearance from the blood in mice proceeds with an exponential half-time of approximately 65 minutes. 3) Concentrations of borate in the tissues of normal mice are directly proportional to dosage for at least 2 hours after injection over a range of 18 to 700 μg/g body weight. 4) At equilibrium distribution, studied in nephrectomized mice, borate is uniformly distributed throughout the body water, with a 10 to 20% excess bound in the intracellular compartment. This excess is bound more tenaciously in brain than in other tissues and appears to be responsible for the toxicity of these compounds. 5) Study of the factors determining the concentration ratio between neoplasm and surrounding normal tissues indi-

cates that boron-neutron treatment offers little promise for malignant tumors other than those of the brain.

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Action of Hydrocortisone on Cells in Tissue Culture.* (21011)

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Since it was established that cortisone inhibits production of granulation tissue in experimentally produced wounds(10), much work has been conducted by numerous investigators studying the direct effect of cortisone on cells in tissue culture. The results have been diverse and in some instances have been contradictory. To mention only a few, inhibition of growth of fibroblasts by cortisone acetate in suspension in concentrations of about 200 to 50 μg/ml (reviewed in 4,2,6), and also by doses as small as 0.5 and 0.3 μg/ml(1,4) has been reported. On the other hand, negative results have been obtained with doses as high as 500 μg/ml (reviewed in 4,8). Stimulation of growth of fibroblasts(6), but also inhibition(8) by desoxycorticosterone have been reported.

In view of the above, attempts have been made to ascertain whether an easily reproducible effect of cortisone on growth in tissue culture, if any, could be demonstrated.

Methods. Primary cultures were used since such growth seemed to be more comparable to growth in regenerating wounds than would be the case if older laboratory strains were used which in long generations may have acquired different qualities. When the first experiments appeared inconclusive, it was thought results might be more clearcut, if embryonic extract (EE) was omitted from the culture medium. It appeared unreasonable to add so potent a growth stimulator as EE, to compete with the substance to be tested, particularly since the active principle of EE is unknown. A medium of amniotic fluid alone, without EE, has been found very satisfactory for obtaining adequate growth in control cultures. In all experiments the hanging-drop method on coverslips was used. In one series of experiments cultures of chick embryo tissue were explanted on coverslips in

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