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A New Common Biochemical Property of Tumors Derived from Different Tissues[1]

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Previous investigations from this laboratory have demonstrated that polarographically reducible materials present in the epidermis of the mouse and man and in the liver and muscle of the mouse are structurally altered when these tissues become malignant (*1–3*). The reducible materials also absorb in the ultraviolet. Evidence of an alteration in the structure of the reducible material in the malignant transformation of epidermis to squamous-cell carcinoma was given by differences in the half-wave potentials and in the absorption characteristics in the ultraviolet of the material from epidermis as compared to that from the carcinoma (*3*). The data presented in this report further substantiate our previous results on a qualitative chemical change in carcinogenesis, and they also show that the tumors examined have a common biochemical property resulting from this alteration.

Methods for the extraction and partial purification of the reducible materials have been given (*3*). Briefly, the tissues were extracted with mixtures of alcohol and peroxide-free ethyl ether, and the total lipid thus obtained by evaporation of the solvents was re-extracted with dry ether, filtered, and the ether removed on a steam bath. Then the acetone soluble fraction of the total lipid, which contained the reducible material, was further fractionated by partitioning it between alcohol, acetone, and water saturated with petroleum ether against the latter saturated with alcohol, acetone, and water. The polarographically reducible material obtained in this manner represented 0.01–0.02% by weight of the fresh tissue. Then nonreducible compounds containing phosphorus were precipitated from the partially purified material in an alcohol-water mixture with calcium chloride (*3*). The latter was spun down at 0° C at 2,500 rpm, and the supernatant was dried at 56°–60° C in a vacuum oven. The dry residue was then treated with 4–6 ml ice-cold water, from which a colored nonreducible substance was separated by centrifugation at 0° C. If the reducible material at this stage was highly colored, much of the colored material could be removed by several extractions with 10-ml portions of peroxide-free ether. The reducible materials thus obtained are light-yellow in color, hygroscopic, soluble in water, alcohol, *N* butyl alcohol, *N* amyl alcohol, and only slightly soluble in nonpolar solvents. The materials are dialyzable through cellophane, stable to heat (steam bath), to storage at 0° to 4° C for a period of months and to oxygen. They appear to be nonprotein.

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Some of the polarographic data obtained from the reducible materials are of interest since they may aid in determining whether the reduction is reversible, and in establishing the number of electrons involved in their reduction (*4*). Although the first wave of the double wave of the material from normal and hyperplastic epidermis (Table 1) appeared to be diffusion-controlled, since the diffusion current and the half-wave potentials were independent of the buffer used at constant pH, from pH 4.0 to 7.2, the relationship between i_d, the diffusion current, and h, the height of the mercury reservoir, was determined. For this experiment the purified material from hyperplastic epidermis was dissolved in 1.5 ml dioxane, 1.5 ml citrate buffer (0.1 *M*) of pH 3.16 (final pH, 4.2), and sufficient tetrabutylammonium iodide was added to make the solution 0.1 molar. The results are shown below:

h(Hg)	i_d(μA)		$i_d/h^{1/2}$
40.5	1.68		0.264
50.5	1.80		0.253
60.5	1.99		0.256
70.5	2.19		0.263
		Average	0.259

Since $i_d = Kh^{1/2}$, the reaction at the dropping mercury electrode is diffusion-controlled (*4*). Similar data were found for the material from the squamous-cell carcinoma.

The diffusion currents and the half-wave potentials were determined on the material from normal epidermis at 2°, 15°, and 25° C in citrate buffer, dioxane, and tetrabutylammonium iodide mixture of pH 6.4, and from liver in the same mixture buffered at pH 5.2 at 2°, 25°, and 40° C. From a plot of the diffusion current against the temperature, the slope of the straight line for the material from normal epidermis gave a temperature coefficient of 1.4%/degree; that from liver was 3.0%/degree. These coefficients are of the same order of magnitude as that of normal diffusion currents (*4*). Furthermore, the half-wave potentials were independent of the temperature, which may indicate a reversible reaction at the dropping mercury electrode (*4*).

In another set of experiments the materials from normal epidermis, squamous-cell carcinoma, liver, hepatoma, and rhabdomyosarcoma were polarographed as previously described (*2*) and log $i/(i_d - i)$ was plotted against E_{de}, the potential at the dropping mercury electrode (*4*). The reciprocal of the slope of the straight lines from the materials from these tissues

TABLE 1

POLAROGRAPHIC AND ULTRAVIOLET ABSORPTION DATA ON SEVERAL TUMORS AND THEIR NORMAL HOMOLOGOUS TISSUES

Tissue	Half-wave potentials, $E_{1/2}$ (v)*	Diffusion current, i_d/mg material (μa)	Absorption maximum (mμ)	Extinction coefficient (ε)	$\frac{\varepsilon}{i_d}$
Normal epidermis	−1.40	0.86	282	7.90	9.24
Hyperplastic epidermis	1.39	.57	282	5.73	10.05
Muscle	1.28	.57	260 (Below pH 4.0)	3.32	5.84
Liver	1.28	.50	260	2.94	5.88
Squamous-cell carcinoma	1.28	.33	260	6.43	19.5
Rhabdomyosarcoma	1.28	.23	260	5.49	21.0
Hepatoma	−1.28	0.13	260	2.38	21.8

* Versus the saturated calomel electrode.

at pH 6.5–6.82 was 0.050 v–0.072 v, with an average of 0.058 v, which is in good agreement with the theoretical value of 0.059 v for a one-electron transfer. The half-wave potentials of the materials from the tissues measured from the plot of log $i/(i_d - i)$ against E_{de} were in excellent agreement with those determined experimentally.

A summation of the data on the reducible materials in some normal tissues and in the tumors derived from them is given in Table 1. The half-wave potentials, characteristic constants for any polarographically reducible substance or for a group of compounds under controlled conditions of electrolysis, in volts versus the saturated calomel electrode, were those obtained in buffered solutions at pH 4.13–4.20. The half-wave potential of the reducible material from normal and hyperplastic (methylcholanthrene-treated) epidermis is about 100 mv more negative than that of the substances from muscle, liver, or from the tumors. The half-wave potentials of the reducible materials vary directly with pH up to pH 7.0 in such a manner that this difference is maintained (*3*); hence only the values at pH 4.13–4.20 are shown. The polarographic waves disappear above pH 8.0 for the reducible materials for all the tissues examined.

In the third column of Table 1 is shown the amount of reducible material, expressed as diffusion current, a quantitative measure of the amount of the materials present, in μa/mg reducible material. (The amount of reducible material was determined on an aliquot of the total sample in vol of 2 ml.) In all cases the tumors contain much less than the tissue of origin. The materials also absorb in the ultraviolet (col. 4); the material from normal and hyperplastic epidermis absorbs maximally at 282 mμ, and the absorption is pH-dependent (*3*); that from muscle absorbs maximally at 260 mμ only when the pH is below 4.0, and that from liver absorbs maximally at 260 mμ from pH 1.5 to 12.0. The material from the tumors absorbs maximally at 260 mμ, and the absorption like that of liver is pH-independent. The absorption maxima of the above samples were measured in alcohol at approximately pH 5–6 (unbuffered) except for that from muscle, which was measured at pH 1.5 in a buffered solution. The extinction coefficients of the materials from the normal tissues and from their respective tumors are not too different except for that from the rhabdomyosarcoma, which is nearly twice that of muscle.

The ratio, extinction coefficient ε : diffusion current i_d, for the material from the tumors is nearly the same value of about 20 : 21, which is much greater than that of the materials from the tissues of origin. The increase in this ratio in the tumors is due to a reduction of the diffusion current, and also to an increase in the value of ε in the muscle tumor. Therefore, the materials in the three normal tissues are different, as indicated by their polarographic behavior or by their absorption characteristics in the ultraviolet, yet the tumors have the common property of a low diffusion current and a constant ratio of ε/i_d. The results presented in Table 1 are the averages of many determinations.

The pronounced difference in polarographic behavior and absorption characteristics in the ultraviolet of the material from epidermis as compared to that from the squamous-cell carcinoma greatly facilitated the discovery of the qualitative chemical change in carcinogenesis. Hence the value of epidermis for the chemical study of carcinogenesis, conceived some years ago by Cowdry (*5*), as the tissue of choice, becomes more apparent.

The reducible materials used in these experiments were not pure. However, the data indicated that polarographic reducibility and absorption in the ultraviolet were properties common to each of the materials present in muscle, liver, and epidermis. Proof of this correlation was necessary because in the malignant transformation of epidermis to squamous-cell carcinoma there is a simultaneous alteration in both polarographic behavior and in absorption characteristics in the ultraviolet of the material which might conceivably result from the alteration of two different compounds. In the transformation of muscle to rhabdomyosarcoma there appears to be only an alteration in the property of absorption in the ultraviolet, or conceivably a change in only one compound. Finally, in the liver-hepatoma system, there appears to be no qualitative chemical change in either property. In other words, if two compounds are involved in the transformation of these normal tissues to malignancies, both are altered in epidermis when it becomes malignant, one in the transformation of muscle to rhabdomyosarcoma, and none in the formation of hepatoma from liver.

Proof that reducibility and absorption in the ultraviolet were common to each of the materials in the

normal tissues was achieved by the countercurrent distribution method of Craig *et al.* (*6*). For this purpose about 10–20 mg of the partially purified reducible materials were dissolved in 10 ml water saturated with *N* butyl alcohol to which was added in a separatory funnel (glass-stoppered) 10 ml butyl alcohol saturated with water. Countercurrent distribution was then carried out using 9 separatory funnels. Equilibrium of the reducible materials in both phases was obtained by inverting the funnels 40 times. Then the water and butyl alcohol layers were collected separately from funnels 1 to 8 and from the top layer from funnel 0 into 50-cc Erlenmeyer flasks. The samples were dried in a vacuum oven at 55°–60° C. The dry material in each flask was then dissolved in 95% alcohol, and an aliquot was used for the determination of the optical density in a Beckman spectrophotometer at the wavelengths given in Table 1. Suitable aliquots from the alcoholic solutions were then dried *in vacuo* at 55°–60° C for polarographic analysis as previously described (*1*).

Since the reducible materials will be distributed in the two phases according to their distribution coefficients, if polarographic reducibility and absorption in the ultraviolet are properties common to one compound, both properties should have the same distribution pattern. This is exactly what we find for the reducible material from epidermis, the distribution pattern of which is shown in Fig. 1. The sum of the

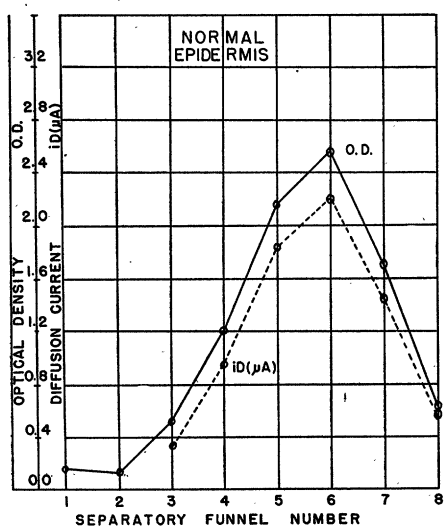

FIG. 1. Countercurrent distribution pattern of reducible substance from epidermis.

optical density of both layers, measured at 282 mμ, and the sum of the diffusion current of both phases are plotted against the number of separatory funnels. Maximum concentration of the reducible material occurred in funnel 6, with a good correlation between optical density and diffusion current in funnels 4–8. This correlation is perfect in all funnels in the water layer, but not in funnels 1–3 in the butyl alcohol phase. This lack of correlation in the latter solvent is due to the presence of a substance which is very soluble in butyl alcohol and which absorbs nonspecifically between 230 mμ and 260 mμ. Some of this material is also present in the water layer in funnels 1–3 and thereby prevents a good correlation in these funnels. The absorption in the ultraviolet was measured only at 282 mμ, since the shape of the absorption curves in both solvents in funnels 3–8 was about the same. The solubility of the reducible material from epidermis was greater in the water layer than in the butyl alcohol layer. Since the properties of polarographic behavior and absorption in the ultraviolet are distributed in a similar fashion in each layer, and are directly proportional to the solubility of the material in each phase, these properties must be common to one compound.

The reducible material from mouse liver was subjected to countercurrent distribution in the same manner as for epidermis. The material was concentrated maximally in funnels 3 and 4 with a good correlation between optical density and diffusion current in funnels 2–5 in each solvent. Some liver samples have shown a slight dissociation of the portion of the reducible material absorbing at 260 mμ in water phase, but never in the butyl alcohol phase. The material was slightly more soluble in the butyl alcohol layer, with respect to both measurements. The reducible material from muscle had a distribution pattern similar to that of liver with a good correlation between optical density and diffusion current in funnels 2–5 in each solvent. The solubility of the reducible material was slightly greater in the butyl alcohol phase. Furthermore, the material from muscle did not absorb at 260 mμ unless the pH was below 4.0. Since the properties of polarographic behavior and absorption in the ultraviolet are distributed in a similar fashion in each layer, and in proportion to the solubility of the material, these properties must be common to the materials from liver and muscle.

The absorption curves of the materials from muscle and liver in both solvents in funnels 2 to 5 were quite similar, so the optical density was measured only at 260 mμ. This was also the case for the materials for the tumors which absorb maximally at 260 mμ, and there was no essential change in the shape of the absorption curves at various pH levels. Furthermore, with the exception of the material from epidermis, the absorption maximum is pH-independent, muscle not absorbing above pH 4.0.

The material from the tumors was subjected to countercurrent distribution in the same manner as for the normal tissues, and the results for the material from the squamous-cell carcinoma are shown in Fig. 2. It is now apparent that the property of reducibility in both layers has concentrated in funnels 3 and 4, whereas that portion of the material absorbing at 260 mμ is concentrated maximally in funnel 6. Analyses of each phase showed that the distribution and solubility of the reducible portion of the material in both phases was about the same, whereas that portion of the material absorbing at 260 mμ was nearly

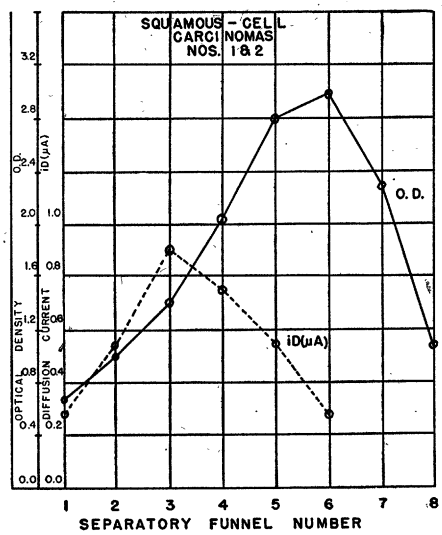

FIG. 2. Countercurrent distribution pattern of reducible substance from the squamous-cell carcinoma.

twice as soluble in the water layer as in the butyl alcohol layer. The material from the rhabdomyosarcoma and hepatoma showed about the same distribution and solubility in the two solvents as that from the squamous-cell carcinoma. These data demonstrate that the reducible material in the tumors has been cleaved, resulting in a common property of the tumors examined.

Since the fraction of the molecule absorbing at 260 mμ was about twice as soluble in water as in butyl alcohol, further evidence of cleavage was obtained by partitioning the materials between 5 ml *n*-amyl alcohol and 5 ml water. The separatory funnel containing the solvents was inverted 50 times to insure a good distribution. After complete separation of the phases, analyses of each were carried out as before. The results of this experiment are shown in Table 2.

From the data in Table 2 it is apparent that the reducible material of epidermis has a much greater solubility in water than in amyl alcohol, with a good correlation between optical density and diffusion current. The materials from liver and muscle have about equal solubility in both phases with respect to these properties. On the other hand, the cleaved portion of the material of the tumors, including a connective tissue sarcoma, absorbing at 260 mμ has about twice the solubility in the water phase as in the amyl alcohol phase, whereas the solubility of the reducible portion of the material, the amount of which is greatly reduced from that of the tissue of origin, is only slightly greater in the water layer. Hence these data on the substance from the tumors confirm those obtained by countercurrent distribution.

TABLE 2

DISTRIBUTION OF THE REDUCIBLE SUBSTANCES IN AMYL ALCOHOL AND WATER

Tissue	i_d water / i_d amyl alcohol	OD water / OD amyl alcohol
Epidermis	4.65	4.86
Liver	1.11	1.29
Muscle*	1.16	1.04
Squamous-cell carcinoma	1.09	1.91
Hepatoma	1.29	1.67
Rhabdomyosarcoma	1.18	2.26
Sarcoma,† connective tissue	1.19	2.07

* At 0° C.
† Originally induced by methylcholanthrene.

Proof that the reducible portion of the material in the tumors has resulted from cleavage of the parent material present in the tissue of origin is given by the following common polarographic properties of both: (1) The half-wave potentials, except epidermis, and their dependency on pH are the same; (2) the polarographic waves disappear above pH 8.0; (3) iodide ion or some other property of tetrabutylammonium iodide, the supporting electrolyte, is necessary for their reduction; (4) a one-electron transfer is involved in their reduction.

Since the properties of polarographic reducibility listed above are the same for the materials as found in the normal tissues and for the cleaved material in the tumors, the reducible portion of the material and that part absorbing at 260 mμ must have come from

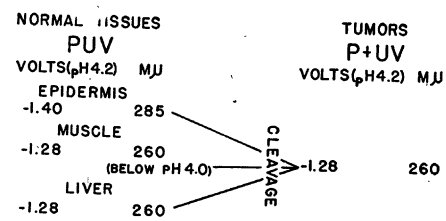

FIG. 3. Graphic presentation of changes in reducible substances when epidermis, muscle, and liver become malignant.

the parent materials. Further evidence that this is true is given in the following data for both the parent materials in the normal tissues, except epidermis, and for the cleaved material in the tumors which absorb at 260 mμ: (1) The absorption curves have the same general shape with a maximum at 260 mμ; (2) the maximum is not affected by changes in pH (substance from muscle does not absorb above pH 4); (3) there is an increase in the extinction coefficient of about 10% at pH 1.3–1.5; (4) there is a similar ratio of maximum at 260 mμ to minimum at 240 mμ; (5) there is a decrease in this ratio (4) at pH 12.0. A more rigorous proof of this correlation will come from infrared spectroscopy and other techniques.

The manner by which the tumors cleave the reducible materials as present in the tissues of origin has not yet been investigated. One might suspect that cleavage has resulted enzymatically, in which case the enzymes responsible for splitting the reducible materials would be either absent or inhibited in the normal tissues. Since the tumors studied are derived from ectoderm (epidermis), endoderm (liver), and mesoderm (muscle), a survey of tumors of other organs

seems advisable to ascertain whether the change in the reducible materials is a general phenomenon.

The evidence presented shows that polarographically reducible materials that are characteristic for epidermis, muscle, and liver are cleaved when these tissues become malignant. A graphic presentation of these changes is shown in Fig. 3. Since the materials are reducible polarographically (P), and since they absorb in the ultraviolet (UV), let PUV stand for the materials as present in the three normal tissues, for both properties are common to each material. Differences in the polarographic and absorption characteristics (or PUV) in the materials from epidermis, muscle, and liver are given in the half-wave potentials and in the absorption maxima. In spite of the fact that these differences exist in the normal tissues, in tumors derived from the latter, cleavage of the parent material, PUV by the tumors into $P + UV$, or two distinct components, has occurred. The half-wave potentials and the absorption maximum of the cleaved products in the tumors examined are the same. In other words, cleavage has resulted in a common biochemical property of these tumors. The nature of the alteration of the reducible materials, particularly in epidermis and also in muscle, suggests that they are altered antecedent to, or concomitant with, cleavage in malignancy. If the alteration of these materials is directly associated with the process of malignancy, a rational approach to the chemotherapy of cancer might follow.

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Comments and Communications

Scientific Manpower

AN ABSTRACT of a report entitled "Why Not a Department of Science and Technology within the Department of Defense?" appeared in the January 1951 issue of the *Chemical Bulletin*. This report outlined a plan for the efficient integration of our military research efforts into a single department concomitant with effective utilization of scientific manpower, facilities, and funds. It is of interest that the Canadian Ministry of Defence has been so organized since 1946.

We believe that the plan is consistent with the resolution on scientific manpower passed by the Council of the American Association for the Advancement of Science December 29, 1950.

As individuals at present actively engaged in research and development in the fields of chemistry, engineering, mathematics, and microbiology who also saw active military service in World War II, we urge that this proposal be given serious consideration.

The following excerpts have been taken from the above-mentioned report:

National safety is dependent on the successful operation of the Department of Defense, which, in turn, is determined by the efficiency with which our natural advantages are employed and detrimental factors eliminated. In terms of manpower, this means that our resources of talent and training must be recognized and made use of under conditions most conducive to the productiveness of the men concerned. Timely utilization of the work potential of men expert in their chosen professions (a factor of considerable importance to morale) may do much to offset any numerical advantage in manpower possessed by potential enemies.

Such reasoning is particularly applicable in the accomplishment of scientific and technical work, which is increasingly important to military operations. Conservation of critical natural resources can be augmented by early research, leading to the more efficient employment of present supplies, and to the development of adequate substitutes. Weapon superiority can only be achieved by constant research and development both in design and in tactical employment. With few exceptions, improvements in the design of weapons and equipment have come in the period preceding war.

It follows that activities and direction in the fields of science and technology are of direct concern to the national defense in times of peace as well as in wartime.

. . . a Department of Science and Technology should be established within the Department of Defense. Let this Department of Science and Technology be headed by a Secretary equal in status to the Secretaries of the Army, the Navy and the Air Force.

All of the research activities and installations now under the jurisdiction of the military services should be assigned to the Department of Science and Technology, making provision for the closest liaison between the Department of Science and Technology and the other three branches of the Department of Defense. The latter would then be free to concentrate on their primary objectives of combat, procurement and supply, assuming only the responsibility for the final development and field testing. . . .

. . . The establishment of such a department would offer the following advantages:

1. Integration of military research on a national level, avoiding duplication of effort and expenditure.
2. Assurance of technically competent superiors to direct scientists, resulting in greater efficiency and greater attraction for competent scientists to peacetime association with the Department of Defense.
3. Operation of a Scientific Personnel Selection Board which would, in an emergency function in the—
   a. Selection of personnel for military research and development.