the 12 species varied from 0.80 to 10 kr.

Figure 1 shows the lethality data for the herbaceous species (*1*) and the new data for the woody species, and Fig. 2 shows the exposures necessary to produce severe growth inhibition, each plotted against the interphase chromosome volumes for the various herbaceous and woody species. In both cases woody species are much more radiosensitive than herbaceous species of comparable interphase chromosome valume. A statistical test indicated that the differences were significant at the 5 percent level.

In Fig. 1 the actual slopes of the regression lines are used but none of the slopes discussed here deviated significantly from −1; all other lines were drawn with a slope of −1 for easier comparison. Since the slopes are essentially −1, the product of the two variables should approach a constant as shown for 16 acutely irradiated herbaceous species (*1*). When exposure is expressed as energy absorbed per unit volume of tissue, the product of the two variables is the total energy absorbed per interphase chromosome at specified exposures and volumes (Table 1). The energy-absorption calculations are based on an average value of 32.5 ev per ion pair and 1.77 ionizations per cubic micron of wet tissue per roentgen. (*4*, Table 2). From a graph of the energy absorbed per chromosome at a lethal exposure against the interphase chromosome volume a regression line was obtained with a slope approaching zero (−0.00453); thus the same principle holds for the sensitivity of woody plants as previously reported for herbaceous species (*1*), that is, the energy absorption per chromosome approaches a constant. A summary of the sensitivities (−1 slope) and the energy absorption (0 slope) for woody species is given in Fig. 3 along with means and standard deviations.

The energies per chromosome required to produce each of the three end points used in the irradiation of the woody plant species were calculated and compared with those of the herbaceous species (*1, 2*). The average amount of energy absorbed per chromosome required to produce lethality in woody plants (1453 kev) is 39 percent of that required for the herbaceous species (3711 kev). Comparable ratios hold for the average amount of energy required to produce severe growth inhibition (997 kev for woody species, 2331 kev for herbaceous species) and slight growth inhibition (534 kev for woody species, 1070 kev for herbaceous species). On this basis the woody species are much more sensitive than the herbaceous species. The cause of this difference is not yet understood.

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Serum Protein Electrophoresis in Acrylamide Gel: Patterns from Normal Human Subjects

Abstract. *Technical improvements in the method for vertical acrylamide gel electrophoresis (Raymond) have resulted in improved resolution and reproducibility. In a survey of approximately 200 normal human serums, ten lines were found to be common in all specimens. Interposed with these constant lines and in addition to known variations in the pattern reflecting haptoglobin and transferrin types, at least one line followed that of albumin and three followed that of transferrin; the presence or absence of these varied from individual to individual. Based on these variations, the electrophoretic patterns of normal humans may be divided into at least 12 discrete groups.*

Serum protein electrophoresis performed in a gel medium, either starch (*1*) or polyacrylamide (*2*), permits the demonstration of approximately 20 specific protein bands, in contrast to the five or six observed on other supporting media. The question arises whether the distribution of serum proteins studied by high-resolution techniques would give rise to patterns as variable as, for example, finger prints, or whether the electropherogram consists of certain constant features with discrete variations superimposed thereon. The fact that transferrin and haptoglobin, two of the chief features of the protein electrophoresis performed on gels, occur only in several discrete genetically controlled variants suggests that many, perhaps all, of the serum proteins are so controlled. We have made a survey of approximately 200 normal serums employing several modifications to the method of Raymond (*3*), and have found additional evidence for the concept that the serum protein electrophoretic diagram consists primarily of several definable proteins.

In these studies a 5 percent acrylamide gel was used. The gels were prepared before each run from the following refrigerated stock solutions: (*A*) Acrylamide, 20 percent: acrylamide 380 g, *N-N'*methylenebisacrylamide 20 g, dissolved in water to 2 : 1; (*B*) DMAPN-buffer: dimethylaminopropionitrile (DMAPN) 8 ml, buffer (20X, described below) 100 ml, and water to 250 ml; (*C*) ammonium persulfate: 0.2 percent in water, prepared fresh each week. To prepare the gel, 40 ml of acrylamide stock (reagent *A*), 20 ml of DMAPN-buffer (reagent *B*), 80 ml of persulfate (reagent *C*), and 20 ml of water were mixed and poured into the cell just before the run. The buffer contained tris(hydroxymethyl)aminomethane, 431 g; disodium ethylenediaminetetraacetate, 37 g; and boric acid, 220 g in 2 liters (*p*H 8.3–8.4), and was diluted 20 times before use in the electrode vessels. The concentration of boric acid in this buffer is higher than that used by others (*4*) and is an important factor in the improved resolution. All reagents were filtered before use and discarded after each use. The buffer was not recirculated. Voltage was applied for 20 minutes before addition of the sample (*5*). The serums used were obtained from normal, healthy blood-bank donors and were stored not longer than 24 hours at 4°C. All specimens were clear and free of hemolysis. Fifteen microliters of serum were applied to each slot. An eight-place slot former was used.

Water at 0°C (for cooling) was circulated through the cell throughout the run. A potential difference of 200 volts (10 v/cm) was applied which resulted in a current of approximately 25 ma at the end of the run (5 hours). The gels were stained for 1 hour in 1

percent amido black 10B in 7.5 percent acetic acid, and they were destained (overnight) in 7.5 percent acetic acid circulated through a charcoal bath. After destaining was complete, gels were photographed with Polaroid type 42 film with a red filter and scanned on a Photovolt Densicord photometer with reduced slit width and a scanning rate of 1.3 cm/min.

Haptoglobin types were determined separately at the conclusion of the study by a modification of the method of Smithies (*6*).

A representative photograph of a gel is shown in Fig. 1. Two lines appearing before the albumins were a constant feature and are not shown. In the "post-albumin" region (between albumin and transferrin) there are eight lines (numbered 1 to 8 commencing at the albumin end). The first line closest to albumin is most frequently masked by the presence of albumin, and although we are certain of its existence we have not been able to make a reasonable estimate of its frequency. All the specimens that we have studied show lines 2, 3, 5, 6, 7, and 8 in the "post albumin" region. The common identity of these lines in each specimen has been established as follows: (i) In a given gel, the migration of a given line is constant through the gel. (ii) A second gel, different in length from the first, shows again a set of seven lines with common migration distance, and this distance may differ in an absolute sense from that found for the earlier gel. Nonetheless, if these two gels contain one or more specimens in common, the common identity of the other lines is established; and (iii) if the migration rate is normalized by the use of transferrin as a marker, that is, if the relative mobility of the line is defined as its migration distance divided by the migration distance of transferrin in the same gel (*7*), then it is found that these lines all possess the same relative mobility (Fig. 2).

Haptoglobin of type 1–1 corresponds almost precisely in its relative mobility to "post albumin" line 7. The appearance of this protein frequently makes difficult the identification of lines 5, 6, 7, and 8 in individuals of haptoglobin type 1–1, but careful study of the scans and photographs reveals that these lines are indeed present.

In the "post transferrin" region (between origin and transferrin) we find variations in the pattern caused by haptoglobin type and by the variable presence of three proteins identified as *a, b,* and *c*. The identity of haptoglobin lines for the various types of haptoglobin was determined by comparisons between serums of known haptoglobin type, by serums with elevated haptoglobins, and by the use of purified preparations of haptoglobins of the three types. It was helpful to determine the migration of these several lines relative to the transferrin and to adopt, as above, a relative mobility unit based on the transferrin migration. Transparent scales constructed from these relative mobilities (Fig. 2) permit the identification on the scans of the lines to be attributed to haptoglobin, and the identification of the three lines *a, b,* and *c* whose relative mobilities were also calculated and are given in Fig. 2.

Three transferrin variants were encountered but are not described here.

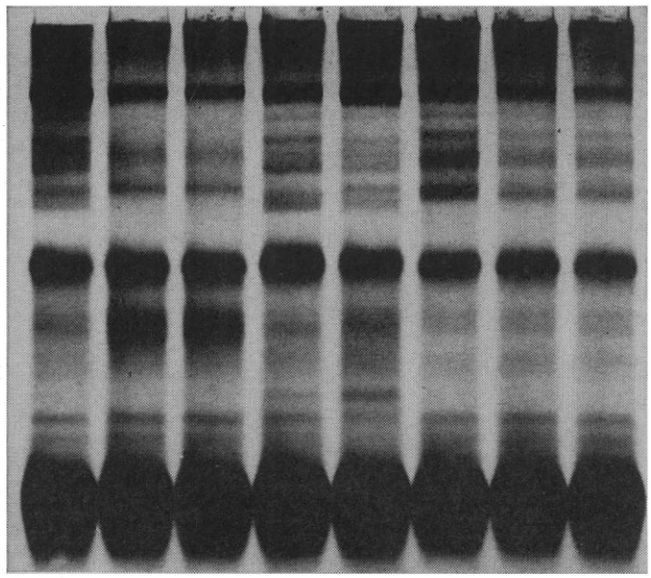

Fig. 1 (left). Electrophoresis patterns of human serums. Reading from left to right, the haptoglobin types are: 2–1, 1–1, 1–1, 2–1, 2–2, 2–2, and 2–2. The first four and the last two positions have proteins *a* and *b*; position 5 has proteins *a, b,* and *c*; position 6 has no *a, b,* or *c*. Line *a* in position 4 is faster than indicated in Fig. 2. Positions 4 and 5 show the presence of "post albumin" line 4. The distance from the origin to the leading edge of the albumin is 10 cm.

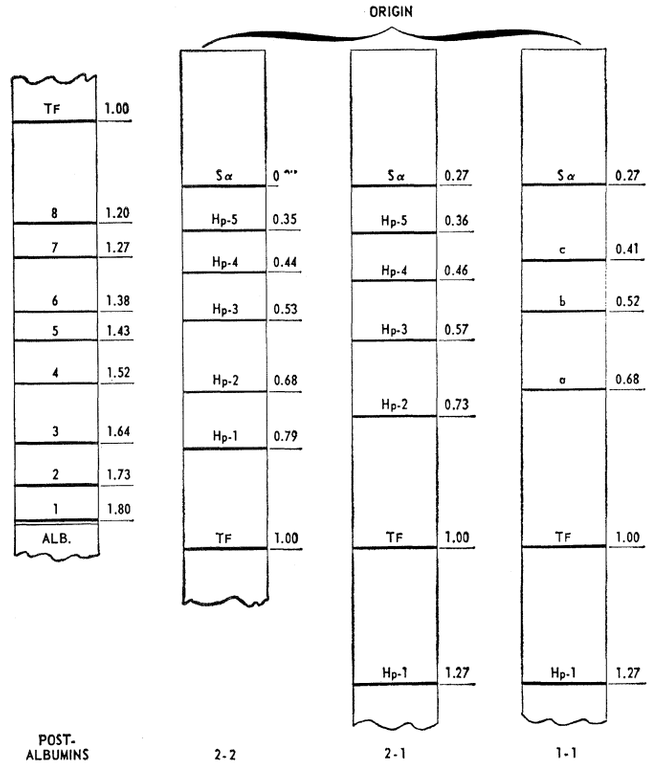

Fig. 2 (right). Schematic representation of the major lines observed in the vertical-gel electrophoresis. The identification of each protein band is given just above the line in the diagram; the relative mobility is given to the right of the line; 2–2, 2–1, and 1–1 refer to haptoglobin types. Additional haptoglobin lines, extending all the way to the origin, are seen when haptoglobin concentrations are above normal. Lines *a, b,* and *c* occur in all three haptoglobin types but have been omitted from the diagram of 2–1 and 2–2 for clarity.

All of the specimens contained the slow α-globulin line as well as a fraction of the material retained at the slot and a diffuse area of γ-globulin.

The frequencies of the variable line 4 in the "post albumins" and lines *a, b,* and *c,* in the "post transferrin" region are given in Table 1. Data on age, sex, race, and the four major blood group types were available on these subjects but could not be correlated in any way with the findings. A second sample of serum, drawn 3 months after the first, was obtained from each of 19 subjects. The results were identical to the results on the earlier sample in each case.

The presence or absence of line 4 was not correlated either with haptoglobin type or with the presence of proteins *a, b,* or *c.* The frequency of occurrence of line 4 (47 percent, Table 1) corresponds to the combined frequency of occurrence of group-specific protein genotype 2–2 and 2–1 (47 percent, *8*), and this correspondence suggests that our line 4 and the Gc 2 protein may be identical. Electrophoretic studies of the Gc series of proteins (*9*) indicate that on starch gel these proteins have mobilities almost as great as that of albumin; studies of the migration of these proteins in the present acrylamide gel system have not yet been performed. There is a distinct possibility, however, that line 4 is Gc 2.

The mobility of lines *a* and *b* corresponds very closely to that for the second and third haptoglobin lines of the type 2–2 (Fig. 2). The near coincidence of these lines makes the presence of *a* and *b* difficult to detect if haptoglobin values are high. We believe, therefore, that the incidence of combinations of proteins *a, b,* and *c* in haptoglobin type 2–2 may be erroneous. Particularly the numerous cases in which neither *a, b,* nor *c* was found in type 2–2 are probably related to the difficulty of detecting these proteins under these circumstances. In addition, the categories *-bc* and *-b-*, not observed in types 1–1 or 2–1, are probably manifestations of failure to detect protein *a* in type 2–2. For these reasons, we feel that evidence for the existence of classes *-b-, -bc,* and no *abc* is inconclusive. In some of the specimens we have examined, it appears that line *a* may move more rapidly than indicated by the average figure given in the table, a suggestion that variants of *a* may occur.

Table 1. Frequency of variant lines in gel electrophoresis by haptoglobin type.

No.*	Haptoglobin type			
	1–1	2–1	2–2	Totals
Total	26	74	80	180
With line 4	11	35	39	85
With *ab-*	21	30	17	68
With *abc*	5	44	13	62
With *-b-*	0	0	12	12
With *-bc*	0	0	7	8
With no *abc*	0	0	31	31

* *a--*, *a-c*, *--c* not observed.

Although difficulty in distinguishing between haptoglobins type 2–1 and 2–2 has been cited (*10*) we have found it relatively easy to make such a distinction in the system employed. All but three of the specimens were correctly identified from the serum patterns. Difficulties in haptoglobin typing appear to arise only when haptoglobin values are substantially below normal.

The presence of hemoglobin in the specimen could influence the pattern by the formation of haptoglobin complexes (*11*). In particular the lines *a, b,* and *c* could conceivably arise by this mechanism. We have studied this question as follows. A random sampling of 32 specimens was investigated for such complexes by performing haptoglobin typing without the addition of hemoglobin. Of these specimens, 23 showed no detectable (benzidine staining) hemoglobin-haptoglobin complex. The remainder showed faint traces of hemoglobin-haptoglobin complexes, which were judged to be beneath the sensitivity of the amido-black stain used in protein electrophoresis. Lines *a, b,* and *c* were found in all 32 specimens, irrespective of the presence or absence of traces of hemoglobin. With fewer than five exceptions the relative mobilities of lines *a, b,* and *c* were the same for all three haptoglobin types, a result not consistent with the view that these lines arise from haptoglobin-hemoglobin complexes. Lines *a, b,* and *c* were also observed in specimens of haptoglobin type 1–1. Laurell (*11*) has shown that the intermediate complexes of hemoglobin with haptoglobin 1–1 migrate between the fully complexed form and the free form of haptoglobin; if such complexes occurred in our study they would be found between line 7 and transferrin and would not be confused with lines *a, b,* and *c.* We believe, therefore, that these three lines exist independently of the formation of complexes between haptoglobin and hemoglobin.

Although the electrophoretic analyses performed in this study were done under rigorously controlled conditions variations in the overall length of the gels has occurred. The variable length, the variations in haptoglobin level, and variations in the incidence of proteins *a, b,* and *c* frequently give rise to circumstances in which the appearance of the gel is misleading with respect to its content. In such cases, accurate measurements are required to determine the nature of the proteins present.

Because the variations in the patterns are independent of one another the minimum number of types of patterns is 12, found by the product of 3 (haptoglobin types), 2 (presence or absence of line 4), and 2 (*ab-* or *abc*). Notable departures from these normal patterns occur in certain diseases. The finding of consistent normal patterns makes more promising the application of this technique for diagnostic purposes.

Note added in proof: A study of serums of known Gc type, as well as of a purified sample of Gc 2–1, kindly made available to us by Drs. Bowman, Bearn, and Kitchin of the Rockefeller Institute, has shown that lines 2, 3, and 4 belong to the Gc series as follows: Gc 1–1, lines 2 and 3; Gc 2–1, lines 2, 3, and 4; Gc 2–2, lines 3 and 4.

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