mental curves, within their range, approximately show this type of change.

It is difficult to explain why Hendler got results so markedly different from the ones described here, and it seems doubtful whether his treatment is generally valid.

JOSEPH KATZ
Institute for Medical Research,
Cedars of Lebanon Hospital,
Los Angeles, California

7 December 1959

The experimental basis for Katz' objection to the straight line relation of F to sample thickness cited in my article [*Science* **130**, 772 (1959)] is essentially contained in six sets of data to which he refers, and which he has kindly supplied me. Two curves for $BaCO_3$ were obtained by Katz by plating on filter paper. These have increasing slopes. One for $BaCO_3$ on filter paper has been in use for many years at the department of physiology, Berkeley, California. These data appear to fit the straight line function perfectly over the whole range from 10 to 80 mg. The curve for the fourth set of data starts with increasing slope, is linear in the central portion, and ends with decreasing slope. Clearly the nature of the curve reflects the individual manner of preparing it. The fifth set of data is described by a curve having a gradually increasing slope which, for the experimental points provided, is adequately described by a straight line.

The last set of data mentioned in Katz' report is for $BaSO_4$ and is the only set published [J. Katz and S. Golden, *J. Lab. Clin. Med.* **53**, 658 (1959)]. I shall address all particular statements to these data which are reproduced in Fig. 1. Although no generality was claimed in my paper for the treatment of S^{35} data, it is gratifying to see that a straight line describes these data with an error which, at its greatest value, is only 2 percent (Table 1). Katz mentions his counting error as being 3 to 5 percent. It must be stressed that no rigid theoretical basis was claimed for the observed straight line relation, and its justification is in its empirical usefulness. In all of Katz' data there is a tendency to increasing slope. I would suggest that at the lower weights of material plated on filter paper certain additional factors which were absent in my preparations (on stainless steel planchets) came into play. First, there is a loss of back scattering. This has been cited by Katz and Golden in their article. Second, the filter paper, being porous and of generally rougher surface than metal, might tend to block radiation from thin samples intimately residing in the texture of this material. Third, it would be difficult, at best, to know if a thin layer of precipitate was completely and evenly spread on the surface of this material. All of these factors would decrease counts observed and hence increase F as the weight was decreased.

Table 1. Comparison of values of F obtained from straight line and from Katz' and Golden's determinations. Straight line equation $F = 0.04m + 0.54$ where m = weight of sample in milligrams.

Mg	Katz' and Golden's value	Value for straight line	Difference (%)*
10	0.93	0.94	1.1
11	0.965	0.98	1.6
12	1.00	1.02	2.0
13	1.04	1.06	1.9
14	1.08	1.10	1.8
15	1.12	1.14	1.8
16	1.16	1.18	1.7
17	1.20	1.22	1.7
18	1.245	1.26	1.6
19	1.29	1.30	1.6
20	1.335	1.34	0.4
21	1.38	1.38	0
22	1.425	1.42	0.4
23	1.47	1.46	0.7
24	1.52	1.50	1.3
25	1.57	1.54	1.9

* The straight line was drawn through all points down to 4 mg. Excluding the less certain values below 10 mg, as Katz and Golden suggest in their paper, would make this small difference tend to disappear.

The "elementary" consideration of the character of self-absorption offered by Katz in his fifth and sixth paragraphs is too elementary. Katz says that when the region of infinite thickness is approached, the count rate (I) becomes constant and the apparent specific activity is therefore directly proportional to weight, with zero intercept. In other words, Katz proposes that at great thicknesses, $F = \frac{R}{I_\infty} m$. He forgets that this is an approximation for $F = \frac{R}{I_\infty} m + b$, where b is small relative to $\frac{R}{I_\infty} m$. For thin samples, Katz uses $F = \frac{R}{I_\infty} m + b$, where as m approaches 0, F approaches b. Thus Katz ignores this intercept value at great thickness but then considers it again at very small thicknesses. By this technique any straight line, $y = mx + b$, would appear to have an increasing slope. For b to equal zero, I must equal I_∞. Both the exponential and hyperbolic treatments state that the true maximum count rate (I_∞) can be achieved only at infinite thickness. Although for our purposes we must accept a discrepancy less than our experimental error between observed and theoretical maximum count rate, we cannot categorically state that such a difference does not exist.

The analysis of F shown in Katz' Table 1 is not at all relevant to the point in question. F is obtained from the best straight line drawn through all of the experimental values. It is easily obtained and can be used to correct experimental data with rather good accuracy. This is true also for the data from which Katz constructed his table. The analysis in Katz' table seems to support the contention that the values at small thicknesses are subject to the errors discussed above, which tend to raise their values.

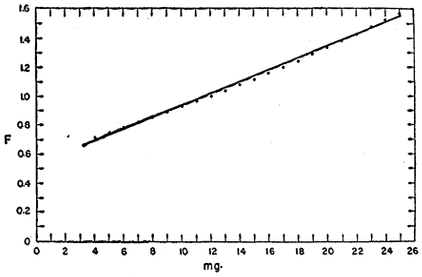

Fig. 1. F values from Katz and Golden plotted against weight of sample over a fixed area.

No rigid theoretical basis is claimed for the straight-line treatment. The obtaining of a straight line is clearly dependent upon the particular technique employed. At the National Institutes of Health, to my knowledge, six individual investigators on a variety of counters have obtained good straight-line relationships. If Katz had known that a straight line $F = 0.04m + 0.54$ could adequately describe his data, he would have saved considerable effort by using it.

RICHARD W. HENDLER
National Heart Institute,
National Institutes of Health,
Bethesda, Maryland

20 April 1960

Induced Somatic Mutations Affecting Erythrocyte Antigens

Abstract. The frequency of inagglutinable erythrocytes was increased in pigeons following total body irradiation and in human polycythemic patients treated with P^{32}. Persistence of the increased levels of inagglutinable cells was observed in pigeons retested at over 200 days after irradiation and in a polycythemic patient retested at 173 days posttreatment. These data provide additional evidence for the mutational origin of the antigen-lacking cells.

Atwood and Scheinberg (*1*) have proposed that the red cells which lack the A antigen are the progeny of bone-marrow stem cells in which mutations have arisen. If these inagglutinable cells arise as a result of mutation they should be increased following irradiation. The procedure for detection of the inagglu-

Table 1. Effect of irradiation on the frequency of inagglutinable cells. Dose is expressed in roentgens to the peritoneum. The inagglutinable frequencies are listed as *F* and the days following irradiation as *D*.

Subject number	Dose	Inagglutinable cell frequencies								
		Initial F	D	F	D	F	D	F	D	F
Pigeon cell carrier, x-rays										
930	0	0.0100	33	0.0090	34	0.0098				
954	500	0.1080	40	0.3370	109	0.2150	204	0.2000	432	.2411
2943	630	0.0460	51	0.3550	126	0.2520	221	0.1700		
986	745	0.0076			104	0.0360	208	0.0128		
921	818	0.0810	40	0.1250	*	*				
Human A cell carrier, x-rays										
893	832	0.0011	54	0.0021						
Human A cell carrier, γ rays from 4π cobalt source (9)										
951	101	0.0028	48	0.0026	149	0.0025				
926	500	0.0014	46	0.0180	120	0.0042	192	0.0050		
908	1004	0.0010	49	0.0030			243	0.0030†		

* Refer to text. † *P. lunatus* extract prepared at later date than extract used initially.

tinable frequency has been described by Atwood and Scheinberg (*1*).

The inagglutinable frequencies before and after irradiation are presented in Table 1. The results were obtained at various times and some represent the first retests of the inagglutinable frequency. Isotope-dilution experiments with pigeons 921, 930, 954, 986, and 2943 were carried out with pigeon cells as carrier, whereas all the other experiments were carried out with human A cells as carrier (*2*). The inagglutinable levels with pigeon cells used as carrier are higher both before and after irradiation than the levels observed with human A cells used as carrier (*3*). Bird 2943 was irradiated while it was lying on its side under the x-ray beam but all other birds given x-ray exposures were kept in a lucite cylinder which was rotated to provide uniform total body exposure (*4*). The dose in roentgens in air (in parentheses) for the various birds is as follows: 954 (700), 2943 (1000), 921 (1023), 986 (945), 893 (1040), 908 (1224), 926 (610), and 951 (120).

Bird 921 was re-bled 7 days after the first retest and showed a fourfold increase in the number of inagglutinable cells. After 61 days the frequency was indistinguishable from the pre-irradiation frequency. These observations suggested that the observed increase in inagglutinable cells was due merely to an increased frequency of young cells arising as a consequence of bleeding. Numerous experiments were carried out which demonstrate that the inagglutinable cells are not merely young cells. Bone marrow cells were found to be reactive with *Phaseolus lunatus* extract and conformed to the red-cell antigen type. Young cells are therefore reactive with *P. lunatus* extract. *Phaseolus lunatus* inagglutinable cells isolated from a population of reactive cells were found to be identical in their reactivity with the initial cell population in that both selected and unselected cells were reactive with two seed extracts and both failed to react with three other seed extracts. With respect to the isolation medium, *P. lunatus* extract, the unselected cells were reactive, whereas the selected cells did not react. Therefore, the inagglutinable (selected) cells are specifically nonreactive with *P. lunatus* extract. As can be seen from the isotope dilution experiments with bird 930 (Table 1), bleeding, by itself, does not explain the increase in the frequency of inagglutinable cells following irradiation.

Experiments were also carried out on a human A_2 polycythemic patient (AG) treated with 2 mc of P^{32} (*5*). The use of the pretreatment value is precluded by sampling error of the initial count, but the frequency of inagglutinables obtained 7 days posttreatment was .0021; 55 days later it was .016; at 97 days, .018; and at 173 days, .0155. This remarkable increase has remained fairly constant for the period of testing. Atwood and Megill (*6, 7*) have obtained results on the inagglutinable frequency in two A_1 patients given 4 mc of P^{32}. The "O" cell population which would be comparable to the inagglutinable frequency of patient AG follows. Patient GP: initially, .00074; 8 days, .00044; 28 days, .00048; 68 days, .00090; 140 days, .00315. Patient AR: initially, .00073; 16 days, .0018; 34 days, .0007; 50 days, .0013; 70 days, .0016; 170 days, .0024.

These constitute definite increases in the "O" level following irradiation, but the variation between individuals precludes a direct comparison with patient AG. However, it appears that 4 mc has not resulted in a greater "O" cell frequency than that obtained with 2 mc, analogous with the difference between the median and high doses observed with pigeons given total body irradiation where doses of 1000 r did not result in an increase in the frequency of inagglutinable cells over that of birds given 500 r. The A_2 levels that were observed by Atwood and Megill (*8*) were more erratic; this is attributed to the effect of temperature on the agglutination reaction with *Dolichos biflorus*.

There is a suggestion of selection against the cells with the inagglutinable phenotype at high levels of irradiation which is also evident in the continued decline in the frequency of inagglutinables in pigeons 954, 986, and 2943. However, at over 200 days there is still a higher frequency of inagglutinables in these pigeons than was observed prior to irradiation. The requirement for additional data on the dose-response relationship precludes further speculation regarding the significance of this observation. The possibility that heavy selection against the inagglutinable cells takes place with high doses of radiation implies gross nuclear or cytoplasmic damage as a result of the high levels of irradiation. Only those bone-marrow stem cells which suffer minor damage will continue to produce terminal red cells which are inagglutinable. It is not known whether the changes induced in the bone marrow cells affect primarily the nucleus or the cytoplasm. However, the persistence of an increased level of inagglutinable cells in irradiated pigeons and in the polycythemic patient suggests that it is the nucleus which is primarily affected.

S. L. SCHEINBERG
R. P. RECKEL
Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

References and Notes

1. K. C. Atwood and S. L. Scheinberg, *J. Cellular Comp. Physiol.* **52**, Supp. 1, 97 (1958); *Science* **129**, 936 (1959).
2. Blood obtained from patient HB through courtesy of B. Gibbs, Walter Reed Army Medical Center.
3. S. L. Scheinberg and R. P. Reckel, *Genetics*, in press.
4. The x-ray machine was made available through the kindness of M. E. Jefferson, U.S. Department of Agriculture, Beltsville, Md.
5. Blood samples were made available through the kindness of Dr. Snyder, Arlington, Va.
6. K. C. Atwood and Donald Megill, "Erythrocyte automosaicism in polycythemics treated with Phosphorus-32," abstract in *Science* **130**, 1411 (1959).
7. Appreciation is expressed to K. C. Atwood, University of Chicago, for use of data presented here and for reading this manuscript.
8. The A_2 levels in patient GP: initially, .0076; 28 days, .0245; 68 days, .0160; 132 days, .0980. Patient AR: initially, .0200; 16 days, .110; 34 days, .0400; 50 days, .0140; 70 days, .0190; 170 days, .01.
9. Radiation facility was made available through the kindness of Commander Chambers, Naval Medical Institute, Bethesda, Md.

30 September 1959