flies, whereas the other baits were being visited as before. The dishes were now shifted so that the Diamalt occupied the right-hand position and the originally screened mush bait the center. The flies were again chased away. Once again, as seen in a picture taken 6 min later, the flies chose the previously visited mush bait in preference to the one that had been covered. They also found their way back to the Diamalt bait in large numbers. The dishes were then returned to their original positions and all flies chased away. Five min later a final photograph was made, in which it was once more apparent that the mush previously visited by the flies was more attractive than the sample that originally was screened. However, one could see also that the number of flies on the formerly screened sample had begun to increase.

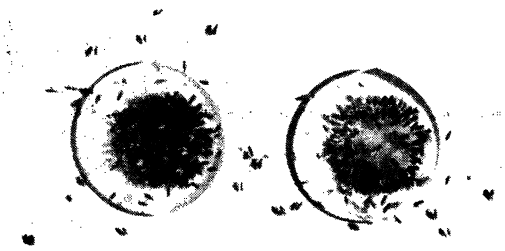

FIG. 1. Effect of previous feeding on attractiveness of proteose peptone to flies. Left-hand dish exposed 20 min, right-hand dish 2 min.

Variants of this test, in which portions of each bait were covered for a time with adhesive tape, and in which the dishes were rotated through various angles or exchanged in position through various sequences, were repeated several times with similar results, proving conclusively that it is visitation by flies and not some other factor that renders either mush or Diamalt baits more attractive. This same effect was observed also with baits initially more attractive than the foregoing materials. In Fig. 1, for example, are shown two dishes, each containing a very attractive mixture of proteose peptone and water. The dish at the left had been subjected to heavy feeding by flies for about 20 min, whereas that at the right had been exposed for only about 2 min.

It is logical to conclude from such data that flies that visit a bait contribute to it some substance which enhances its attractiveness to the species. The nature of this substance is unknown, although we have found that a material attractive to flies and soluble in 95% ethanol, but much less soluble in acetone or ether, can be extracted from the bodies of these insects. Further efforts to isolate and identify this substance, and to determine whether it is identical with that contributed to baits by flies which visit them, are planned.

Meanwhile, the observations outlined above are of obvious significance for the design and interpretation of field experiments on fly attractants. Valid conclusions in regard to the attractiveness of test materials will be possible only when experiments are so arranged as to permit a distinction between attraction exerted by the test compounds and that derived from previous contact with the insects.

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Distribution of Allergic and "Blocking" Activity in Human Serum Proteins Fractionated by Electrophoresis Convection[1, 2]

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The purpose of this investigation was to determine the distribution of allergic, or reaginic, antibodies in human serum proteins. Electrophoresis convection was chosen as the means of fractionation of these skin-sensitizing antibodies not only because of its gentle nature but also because sufficient volumes of fraction are involved to permit complete immunologic and electrophoretic characterization—objectives precluded by the small yields of conventional electrophoresis. Whereas the latter procedure led Newell *et al.* (*1*), as well as Sherman and Seebohm (*2*), to conclude provisionally that reagins for pollen resided in γ-globulin, the factors responsible for "cold" allergy appeared more disseminated. Cooke and collaborators (*3*) later concluded that the γ-globulin activity of individuals allergic to animal dander, pollen, or mold spores was about 10 times *lower* than the corresponding dilution titer of the original serum. Campbell and associates (*4*) were the first to apply the method of electrophoresis convection to the problem. They found that activity was closely associated with the α- and β-globulins in one serum, whereas it appeared to be distributed among all the globulins in another.

In the present investigation, electrophoresis convection has been extended to 7 stages, rather than 3, and the more reliable "passive transfer" procedure of neutralization with allergen *in vitro* has been added to the technique of serum dilution employed exclusively by earlier workers. Sufficient material was also available to do titrations in three normal test subjects, as well as to observe the fractions for stability during a 4½-month period. Furthermore, electrophoretic analysis and chemical assays for protein content were done on each preparation.

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Fractionation. The details of construction and operation of the electrophoresis-convection apparatus, as well as its application to the separation of serum proteins, have been described by Kirkwood *et al.* (*5–7*). A woman highly allergic to all preparations of commercial insulin served as the source of the 70 ml of serum needed for fractionation. At the conclusion of each run, the contents of the upper reservoir were passed through a bacterial, Seitz filter and were set aside at 4° C for future study under the label Top 1 or 2 or 3, etc. "Bottom" solution remaining in the cell was then diluted for the next run. Whereas the first 6 stages were carried out in phosphate buffer of 0.1 ionic strength during 48–51 hr at field strengths ranging from 1.5 to 2.5 v/cm, acetate buffer of the same ionic strength was employed for the 7th run of 73 hr. The pH levels chosen for successive runs were 7.5, 7.0, 6.5, 6.0, 6.0, 5.3, and 5.2. After the last stage, bottom fraction was separated into a globulin and an albumin portion by adding one volume of saturated ammonium sulfate at pH 7. The globulin fraction was further purified by reprecipitation, then labeled BG-7 to distinguish it from the albumin, BA-7.

Electrophoretic analysis was done on a 1% solution of each fraction, pervaporation being necessary in the case of the top and albumin preparations. Barbital buffer at pH 8.6, ionic strength 0.1, and a field strength of about 7 v/cm were employed for the 2-hr runs in the apparatus of Perkin and Elmer, voltage being measured with a potentiometer. Mobilities were calculated (*8*), and the relative area of each component was determined by the use of a planimeter on a projected tracing of the descending pattern. It was then possible to estimate the protein concentration for individual components, since the protein content of each fraction had been established and the contribution of its several components to the total area had been measured.

TABLE 1

ESTIMATED PROTEIN CONTENT OF VARIOUS ELECTROPHORETIC COMPONENTS*
(mg/ml)

Fraction	Assayed protein content of fraction	Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin
Whole serum	70.0	35.0		4.2	19.6	11.2
Top 1	3.1	.06			.1†	2.9
Top 2	2.7	.08			.1	2.5
Top 3	0.8	Trace			< .1	.7
Top 4	0.9	"			.2	.6
Top 5	0.3	"			.1	.2
Top 6	4.2	.4		1.0	2.1	.7
Top 7	8.9	1.9	2.6	1.6	2.9	
Bottom 7	43.0	34.4	4.3	3.0	1.3†	
BA-7	43.0	37.8	(———	5.2	———)	
BG-7	5.6		2.8	2.8		

* Area of component in per cent was multiplied by assay figure for protein content of fraction.
† Estimate unreliable, as β-globulin comprised only 3% of area.

TABLE 2

DISTRIBUTION OF SKIN-SENSITIZING POWER IN HUMAN SERUM PROTEINS AFTER FRACTIONATION BY ELECTROPHORESIS CONVECTION
(As judged by *in vitro* neutralization with allergen [insulin])

Fraction	Total protein (mg/ml)	Insulin required to neutralize fraction		Insulin required to neutralize globulins (u/mg protein)	
		u/ml	u/mg total protein	Total globulins	β-globulin
Whole serum	70.0	35.0	0.5	1.0	1.8
Top 1	3.1	0.25	0.08	.08	2.7*
Top 2	2.7	0.25	0.09	.01	1.6
Top 3	0.8†				
Top 4	0.9†				
Top 5	0.3†				
Top 6	4.2	5.0	1.2	1.3	2.4
Top 7	8.9	7.5	0.85	1.1	2.7
Bottom 7	43.0	1.0	0.02	0.12	0.8*
BA-7	43.0	0.25	0.006	0.05	*
BG-7	5.6	0.5	0.09	0.09	

* Electrophoretic area too small for reliable estimate.
† Sensitizing power almost negligible.

Activity tests. Each fraction was given a preliminary test for sensitizing power, 0.1 ml of undiluted material being injected intracutaneously into a normal volunteer. In 24 hr each site received 0.025 ml of Squibb crystalline insulin containing 4 u hormonal activity/ml. Allergic potency was judged by the size of the wheal-and-flare which promptly developed. Immunologically active materials were subsequently examined in serial twofold dilutions, as well as admixed in undiluted state with an equal volume of insulin in doubling concentrations. One-tenth ml from each tube was then injected into the skin of one of three normal subjects for test with insulin next day, so as to reveal the greatest dilution in which each fraction could transfer sensitivity, as well as the minimal amount of insulin required to neutralize its sensitizing power (*9, 10*). Although the relative potencies of the various fractions were roughly confirmed in two other recipients, it will suffice to present the results of one subject, E.M.M. Only those obtained during the first few weeks of study will be given, since the fractions (but not the original serum) showed decided loss of potency during the 4½ months of observation.

Tops 1–4 were found to contain fractions of the γ-globulin of the original serum, with a mobility spectrum ranging from -1.31×10^{-5} to -1.91×10^{-5}. Tops 1, 2, and 3 consisted almost exclusively of γ-globulins (95–90%). Tops 5, 6, and 7 were the richest in β-globulins, containing 39, 50, and 32%, respec-

tively. Whereas BG-7 was comprised entirely of α_1- and α_2-globulins, fraction BA-7 was 88% albumin, according to planimeter measurements. Table 1 lists the protein content of the several components of each fraction, as calculated from its chemical and electrophoretic analyses, and subsequent tables express activity in terms of potency per mg of component protein.

The conclusion was drawn that activity is associated almost exclusively with β-globulin, this deduction being based on the following analysis. First, albumin and the α- and γ-globulins were excluded from consideration as major contributors of activity, inasmuch as an upper limit of very low order could be put on each of them. Fraction BA-7, for example, contained 88% of albumin but required only 0.25 u insulin/ml for its desensitization (Table 2). This amounts to only 0.006 u insulin/mg albumin. Similarly, Tops 1 and 2 were comprised of 95 and 91% γ-globulin, and carried this same low neutralization requirement. If the activity were attributed exclusively to γ-globulin, its neutralizing power amounted to only 0.08 u/mg, or less.

The α_1- and α_2-globulins were judged by fraction BG-7, which was comprised solely of these two proteins and possessed an activity of only 0.09 u insulin/mg, a finding discouraging to assumption that α-globulins might play an important role. If all the sensitizing power of the whole serum had been referable to its α-globulin component, this would have had to carry a requirement amounting to 8.35 u insulin/mg α-protein. The discrepancy between this and the above observation for BG-7 is inconsistent with the proposition that the α-globulins carry significant activity.

In contrast with the foregoing, high activity was exhibited by Tops 6 and 7, both of which contained large proportions of β-globulin. Making the most liberal allowance for activity of the α-globulins in these fractions, based on BG-7, the neutralizing power

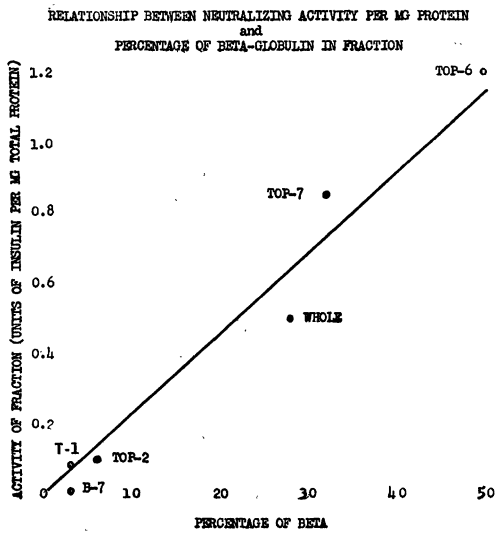

FIG. 1.

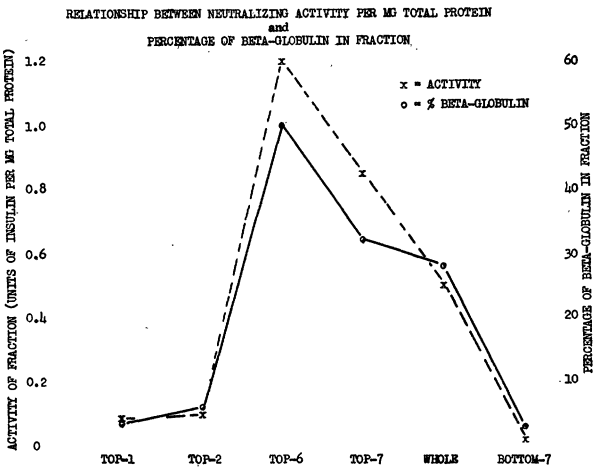

FIG. 2.

of β-globulin/mg was calculated to be 2.4 u insulin for Top 6 and 2.7 u for Top 7. This is over 20 times the maximal potency that can be attributed to either the γ- or the α-globulins.

Further evidence for the hypothesis that activity resides principally in β-globulin is furnished by the observation that the activity per mg determined for Top 1 is roughly 2.7, for Top 2 approximately 1.6, and for whole serum, 1.8—values which can be considered constant within the limits of the assay method employed. (Bottom-7 and BA-7 are not included in this discussion because their β-globulin areas were too small for reliable estimates.) On the other hand, the assumption that reagins are uniformly distributed among all the globulins is untenable in view of the computed activities per mg of total globulin, since they are by no means constant, ranging from 0.01 to 1.3 (Table 2).

In Fig. 1, the activity per mg of total protein is plotted as a function of the percentage of β-globulin in the several fractions. It will be observed that, within the limits of experimental error, the neutralizing power is proportional to the β-content, which is consistent with the hypothesis that activity resides in the β-globulin and that it amounts to approximately 2 u/mg. In Fig. 2 the percentage of β-globulin and the activity per mg of total protein are shown to be precisely correlated. The conclusion to be drawn from the foregoing evidence is that β-globulin possesses a neutralizing activity at least 20 times greater than that of any other protein in the serum. This is confirmed by the serum-dilution results for the various fractions, the order of potency closely following that of the neutralization end points (Tables 2 and 3). Fractions with small dilution ratios possessed low concentrations of β-globulin, as will be noted in Table 1. Tops 1 and 2, for example, contained only about 0.1 mg/ml. The right-hand column of Table 3 shows that the maximal dilutability of a solution containing 1 mg β/ml was approximately 1:10, indicating that the minimal strength for borderline sensitization was

TABLE 3

DISTRIBUTION OF SKIN-SENSITIZING POWER IN HUMAN SERUM PROTEINS AFTER FRACTIONATION BY ELECTROPHORESIS CONVECTION

(As judged by sensitization tests with serial dilutions)

Fraction	Dilutability of original fraction	Dilutability of solution containing 1 mg/ml of		
		Total protein	Total globulin	β-globulin
Whole	256	3.7	7.4	13.2
Top 1	2	0.65	0.66	21.5*
Top 2	2	0.75	0.76	12.5
Top 3	< 1			
Top 4	< 1			
Top 5	< 1			
Top 6	24	5.7	6.3	11.4
Top 7	32	3.6	4.6	11.2
Bottom 7	4	0.09	0.47	3.0*
BA-7	1	0.02	0.19	*
BG-7	2	0.35	0.35	

* Electrophoretic area too small for reliable estimate.

roughly 0.1 mg β/ml, as determined from Tops 6 and 7, which transferred markedly in our subject when employed in their original concentrations. The feeble sensitizing qualities of all other fractions were, therefore, consistent with the concept of β-activity.

It will be remarked that Tops 3, 4, and 5 could not be used in the analysis of β-activity (Tables 2 and 3), since they transferred only questionably in E.M.M. From Table 1 it will be noted that the β-globulin concentration of Tops 3 and 5 approximated 0.1 mg/ml, the figure mentioned above as the borderline requirement. Top 4, with its content of 0.2 mg, should have produced detectable sensitization. Although it failed to do so in E.M.M., it transferred slightly in another recipient, resembling Tops 1 and 2 in potency, as might have been expected from its rather low β-content.

Since the writing of this paper, another serum containing reagins (for ragweed pollen) has been similarly fractionated and studied by the dilution technique. Activity appeared to be distributed through the γ- and β-globulins. The maximal volume in which 1 mg of total protein would still transfer sensitivity ranged from about 1 ml for Top 1, which consisted predominantly of γ-globulin, to approximately 4 ml for Top 7, the β-rich fraction. These results suggest that allergic activity is concentrated in β-globulin. However, it was not restricted to this protein as in the instance of our insulin-reaginic serum.

The pollen-reaginic serum also contained thermostable, or so-called blocking, antibody. Its presence in the fractions was judged by the amount of pollen antigen each could neutralize, heated samples being mixed with graded strengths of pollen extract for subsequent test in sensitized normal skin. Neutralizing activity was found in fractions rich in γ-globulin. Top 1 (consisting 91% of a slow γ-globulin having a mean mobility of -1×10^{-5}) possessed a neutralizing power of about 10 phosphotungstic-acid-precipitable N u pollen/mg total protein. Top 5 (containing 70% of fast γ-globulin, with a mean mobility of -2×10^{-5}) carried an inhibiting activity of over 30 u/mg in two test subjects. Antibody content appeared to be minimal in Tops 2 and 3, which were composed largely of γ-globulin of intermediate mobility. The remaining fractions, comprised almost exclusively of β-globulins, α-globulins, or albumin, showed negligible neutralizing power. We therefore conclude that the thermostable antibody is concentrated in the γ-globulins, with a bimodal distribution. The latter might be attributable to the existence of more than one antibody for the multiple antigens known to be present in pollen.

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Effect of Halogens on the Production of Condensation Nuclei by a Heated Platinum Wire

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Experiments performed in this laboratory show that the presence of small traces of gaseous halogens or halogen-containing compounds in the atmosphere causes a very large increase in the rate at which a heated platinum wire produces condensation nuclei. The apparatus used in these experiments is shown in Fig. 1. Air from the room is drawn through a long filter of fine glass wool, which removes practically all condensation nuclei. This nuclei-free air is then passed through a chamber containing a platinum filament electrically heated to about 500° C and into an apparatus which measures the concentration of condensation nuclei. In this work an automatic condensation-nuclei-measuring device (*1*) was used, but less complicated equipment, such as an Aitken counter (*2*) or a simple expansion chamber, is satisfactory. When the platinum filament was first turned on, a large concentration of nuclei was produced. The formation of these nuclei apparently resulted from surface contamination of the filament, for after a few minutes of operation

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