not pant and have few sweat glands. Saliva production in desert rodents has been seen only under conditions of extreme heat stress, and was interpreted as an emergency reaction which just precedes and momentarily delays thermal death (*8*). The work reported here shows that saliva production in the normal rat occurs not just at lethal extremes but over a wide range of ambient temperatures. This suggests that the evaporation of spread saliva may be as important an element in the rat's defense against hyperthermia as other mechanisms of heat loss.

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Antibody Molecules: Discontinuous Heterogeneity of Heavy Chains

Abstract. *The heavy polypeptide chains of antibody (and of γG-immunoglobulin) molecules show discrete bands on disc electrophoresis. The same bands are present for chains from antibodies of the same or diverse specificities. Individual bands are of different intensities for chains from the different rabbits tested even if the antibodies are directed against the same hapten. The bands appear to represent classes of heterogeneous H-chains of the same size having discrete differences in mobilities with respect to a single charge difference.*

The heavy polypeptide chains (H-chains) of specifically purified antibodies have not previously been resolved into discrete classes. Heavy chains from nonspecific human immunoglobulin G (γG) (*1*) have been reported as showing bands on starch-gel electrophoresis (*2*). We now report that it is possible to resolve the H-chains of specifically purified rabbit γG antibody to hapten and of rabbit γG-immunoglobulin (*1*) into discrete groups by disc electrophoresis in polyacrylamide gel (*3*). The methods used separate H-chains of rabbit γG-immunoglobulin into seven or eight discrete bands in the gel. The H-chains of a specific antibody from a particular rabbit may fall into only a few or into all of these bands. Antibodies of the same specificity but from different rabbits may have different patterns of bands. Furthermore, antibodies directed to different haptens have many of the bands in common. Therefore, structural features associated with the specificity are not of primary importance in the observed electrophoretic-banding behavior of the H-chains.

Whole γG-immunoglobulin, specifically purified antibody to hapten, and γG-immunoglobulin from which antibody had been removed (antibody-depleted γG-immunoglobulin) (*2*) were isolated from normal rabbit serums or from rabbit antiserums to haptens (*4*), and the H- and L-chains were prepared from these (*5*). The preparations of H- and L-chains were concentrated by pervaporation at 5°C and stored in 0.01 to 0.05*M* propionic acid. The H-chains were resolved by disc electrophoresis at *p*H 4.3 in a 15-percent polyacrylamide gel (*6*) by Davis' "alternative" procedure (*3*). The sample solution was prepared by dissolving 120 mg of sucrose in 0.1 ml of stock solution B (*6*) diluted 1 : 8 with water, and to this mixture 0.1 ml of 0.01 to 0.05*M* propionic acid containing 0.1 mg of H-chains was added. One-tenth milliliter of this sample solution was placed on the spacer gel. Electrophoresis was carried out (3 ma for 1 hour) with the cathode at the bottom of the gel. Methyl green was used as the tracking dye (0.5 ml of 1 percent aqueous solution per 300 ml of electrophoresis buffer). The gels were stained with Buffalo black NBR, and the stain was removed in the usual manner (*3*).

The patterns of bands for the H-chain of specifically purified antibody to *p*-azobenzoate hapten (anti-X_p) from two different rabbits (Nos. 2662 and 3351) are shown in Fig. 1 as *a* and *b*. The patterns are remarkably similar with seven bands present in *a* and six in *b*. Band 3 is very sharp in Fig. 1*a* but is not seen in Fig. 1*b*. Band 1 is weak in both patterns. The six bands in *b* appear to be identical with those of *a* as indicated by the pattern *c* for a mixture of equal portions of the two preparations. Each band is as sharp with the mixture as with each separate preparation.

The H-chain pattern of antibody to *p*-azotrimethylphenylammonium hapten (anti-A_p) obtained from a pool of antiserums from several rabbits shows bands (reproduced in Fig. 1*d*) corresponding to bands 2, 4, 5, 6, and 7. Bands 2 and 5 are relatively weak. The H-chains from antibody to *p*-azobenzenearsonate hapten (anti-R_p) from two individual rabbits (Nos. 3287 and 3075) gave patterns (Fig. 1, *e* and *f*) which are similar in that bands in the region corresponding to bands 4 and 5 are relatively diffuse. However, the pattern of Fig. 1*e* shows band 2,

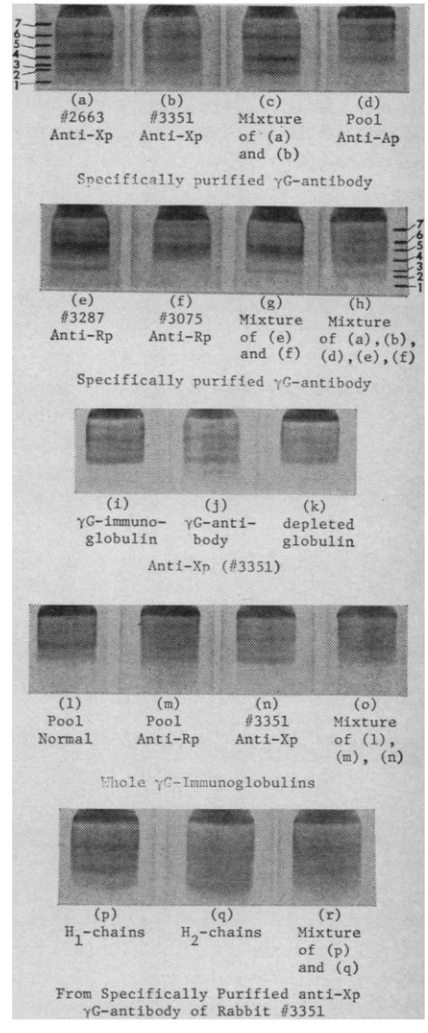

Fig. 1. Disc electrophoresis pattern (*p*H 4.3) of H-chains from rabbit γG-immunoglobulins.

which is absent in Fig. 1*f*, and the relative intensities of bands 6 and 7 differ in Figs. 1, *e* and *f*. The identity of the corresponding bands in Fig. 1, *e* and *f*, is shown in Fig. 1*g*, which is the pattern of a mixture of equal portions of the two anti-R_p antibody preparations. The diffuse band is not altered, and the intensity of band 2 is significantly lower than in Fig. 1*e* because of its absence in Fig. 1*f*.

The identity of the bands of the five individual preparations shown in Figs. 1, *a, b, d, e,* and *f* is shown in Fig. 1*h*, the pattern for a mixture of equal portions of these five preparations. Bands 1 and 3 do not appear in pattern *h* because they did not occur in all preparations and were apparently diluted out in Fig. 1*h*. The rest are of approximately the same intensity, owing to an averaging of their concentrations. The diffuse bands in individual patterns Fig. 1, *e* and *f* appear as bands 4 and 5 in the combined pattern Fig. 1*h*. All five of the preparations contained H-chains which fell into bands 4, 5, 6, and 7; thus these appear to be common although the relative intensities vary between preparations. Band 2, seen in both anti-X_p patterns (Fig. 1, *a* and *b*), is very weak in the anti-A_p pattern (*d*) and is detected in only one anti-R_p pattern (*e*), while band 1 is apparent only in the anti-X_p patterns (*a* and *b*).

Patterns in Fig. 1, *i* and *j* (serums from rabbit No. 3351), show that the H-chains of the whole γG-immunoglobulin from anti-X_p (pattern *i*) and the H-chains of the specific antibody (anti-X_p) from this whole globulin (*j*) fall into common bands. In pattern *i*, the bands correspond to bands 4, 5, 6, and 7. These are also present in *j*. In addition, Fig. 1*j* shows bands 1 and 2, although the former band is very faint. Neither of these bands is detected in Fig. 1*i*, the pattern for the whole globulin, because the antibody molecules from which these bands were derived were of relatively low proportion in the whole globulin.

The H-chain pattern of the antibody-depleted γG-immunoglobulin is shown in Fig. 1*k*. There is a decrease in the intensity of bands 4 and 6, relative to the other bands. This decrease is due to the removal of the antibody which contained most of the H-chains falling into bands 4 and 6. Thus, the integrity of the various chains is maintained through the purification procedure as well as through the other steps leading to the H-chain isolation, and the pattern observed is characteristic of the individual components of the γG-immunoglobulin concerned in each case.

Patterns of Fig. 1, *l, m,* and *n* are for the H-chains of the whole γG-immunoglobulin fraction from normal serum (*l*) from antiserum to R_p (*m*), and from antiserum to X_p (*n*). The same bands are seen, although in different proportions. The pattern of a mixture of equal portions of these three (*o*) demonstrates the identity of the various bands from these patterns.

Two fractions of H-chains are obtained during the separation of the H- and L-chains (light chains) on Sephadex G-100 (*5*). The material in the two peaks derived from specifically purified antibody to X_p from a single rabbit was subjected to disc electrophoresis. The pattern of bands for H-chains in the eluent in the first peak is shown on pattern *p* and that for the eluent in the second peak is shown in *q* while *r* is that of a mixture of the two. The two fractions clearly differ in intensities of the bands although both gave the same bands.

The separation of H-chains into two fractions on Sephadex G-100 (in 1*M* propionic acid) must be due to a difference in size. This can be due to swelling, the degree of aggregation in the two fractions, or both. Ultracentrifugal measurements show a low sedimentation coefficient ($s_{20,w}$) for the H-chains in 1*M* propionic acid at 5°C (*7*). Proteins are known to swell in acid solution (*8*). At least some of the H-chains contributing to bands 4 and 6 are of larger size than are others under the conditions used for separation on the Sephadex, since these bands were more intense in the pattern of Fig. 1*p* (first eluate peak).

If some of the H-chains are aggregated in 1*M* propionic acid, they dissociate enough under these conditions of gel electrophoresis so that all of the protein usually enters and passes through the gel. Furthermore, H-chain patterns obtained under the conditions used here are reproducible, and, for a given preparation, the relative distribution of materials in the bands is not altered either by increasing the amount of sample, changing the length of the spacer gel, or by both. This indicates a high degree of dissociation of the aggregates during disc electrophoresis.

The results obtained here demonstrate a physical heterogeneity of the H-chains of rabbit γG-antibody. The banding is not a direct function of the specificity of the antibody, because antibodies of different specificities give common bands. Moreover, antibodies directed against the same hapten show differences in bands.

The difference in mobility between each pair of adjacent bands is the same. These differences in mobility of adjacent bands are compatible with single charge differences between chains all of essentially the same size. The single charge differences represent differences in primary sequences (*9*). The same argument regarding single charge differences holds for light-chain banding (*10, 11*).

Two other possibilities for the banding observed, besides the single charge differences, are (i) that some of the H-chain bands observed may represent different degrees of aggregation of certain kinds of H-chain; and (ii) that the bands result from different degrees of alkylation of a single type of H-chain during the original reduction and alkylation of the whole antibody. In (i) the observed differences in intensities of banding would indicate differences in aggregation which would be due to differences in the proteins in the individual bands. Possibility (ii) does not seem to be the case since the banding for individual heavy chain preparations is reproducible for samples of the same antibody preparation alkylated at different times. Moreover, the banding observed for H-chains from various sources differs although the degree of alkylation depends on the degree of reduction, and this does not vary much in view of the fact that the reduction is carried out without a denaturing agent such as urea and at a low concentration of mercaptoethanol (*12*). The differences in the amounts of each band in various preparations must thus be due to differences in the heavy chains, otherwise the bandings would be the same for the various preparations.

These results are compatible with the hypothesis that many different types of cells or loci produce γG-antibody (*11, 13*). Each type produces a particular γG-immunoglobulin with a characteristic charge on the H-chains (and L-chains) and certain types respond better to particular antigens than do others. Thus for a particular antigen, H-chains with certain characteristic charge properties predominate because of the preferential antibody formation by certain cells or loci, whereas for another antigen the H-chain could fall into a different distribution

of bands because other cells may be more important in forming the antibody proteins.

In this connection differences in the protein of γG-antibody formed for two different haptens by a single rabbit have been reported (*14*). Also, Sela and Mozes (*15*) showed that the nature of the carrier protein of the antigen affects the protein type of γG-antibody formed against a single hapten. The differences could be due to the production of antibody by different cells.

The differences in gel patterns of H-chains from antibody of the same specificity but from different rabbits could be due to a variation in the relative distribution of these different antibody-producing cell types in individual rabbits.

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Lead-210 and Polonium-210 in Tissues of Cigarette Smokers

Abstract. *Concentrations of lead-210 and polonium-210 in rib bones taken from 13 cigarette smokers were about twice those in six nonsmokers, the polonium-210 being close to radioactive equilibrium with the lead-210. In alveolar lung tissue the concentration of lead-210 in smokers was about twice that in nonsmokers. These differences are attributed to additional intake by inhalation of lead-210.*

Because of the correlation between the smoking of cigarettes and the presence of somatic effects, such as carcinoma of the lung, many studies have been made of the carcinogens present in the smoke. The presence in tobacco of the alpha-emitting and volatile radionuclide ^{210}Po has lead to several studies correlating the distribution and concentrations of this nuclide in the human body with cigarette smoking. Thus Radford and Hunt (*1*) reported that in the bronchial epithelium of a heavy smoker the activities were such as to produce radiation levels of 165 rem over 25 years. On the other hand, Hill (*2*) and Rajewsky and Stahlhofen (*3*) estimated the doses to be less than 1 and 0.15 rem/year, respectively. Hill (*2*) and Ferri and Baratta (*4*) also showed the ^{210}Po concentrations in these and other tissues to be higher in smokers than in nonsmokers.

However, because of the relatively short half-life of this nuclide (138 days), its precursor, the lead-210 with a 21.4-year half-life, is also of interest. This nuclide decays by a weak beta-emission to the 5.0-day ^{210}Bi, which in turn decays by 1.1-Mev beta-emission to ^{210}Po. The data we present demonstrate that, in skeletal tissues, not only are the concentrations of ^{210}Po greater in smokers than in nonsmokers, but in both skeletal and lung tissues of smokers the concentrations of ^{210}Pb are also greater. Moreover, in bone the ^{210}Po is in radioactive equilibrium with the ^{210}Pb.

Lead-210 is also shown to be present in the smoke, an association not unexpected since stable lead is known to occur in smoke (*5*) and Nusbaum *et al.* (*6*) have shown correlation between concentrations of lead in bone and cigarette smoking.

Our measurements were made on rib bones and alveolar lung tissue taken at autopsy (or surgery) from subjects of known smoking habits. Individuals smoking more than ten cigarettes daily were classed as smokers, but in our group only one member consumed less than 20.

Activities were determined by wet-ashing the materials in nitric and perchloric acids, converting the solutions to 0.5*N* hydrochloric acid, and plating the ^{210}Po upon a silver disk. The amount of polonium was determined by counting the disk in a gas-flow internal alpha counter (*7*).

This measurement, along with a replating of the ^{210}Po grown-in for several months from the ^{210}Pb in the original solution, and application of the radioactive parent-daughter relation of the Bateman equations, enabled determination of the activity of each of these nuclides at the time of autopsy or surgery. The average errors at the 90-percent confidence level, based on counting statistics, were about 15 percent for ^{210}Pb and about 20 percent for ^{210}Po.

Concentrations of ^{210}Pb and ^{210}Po in bone are presented in Table 1 along with sexes and ages. The groups are matched by age but not by sex; this mismatch should be examined further, since, as noted earlier (*7, 8*), the skeletal ^{210}Pb content of women appears to be lower than that of men. Within this particular small group of nonsmokers no statistically significant difference exists.

The mean concentrations of both nuclides in smokers, 0.285 pc ^{210}Pb and 0.250 pc ^{210}Po per gram of ash, are more than double those in nonsmokers: 0.135 pc ^{210}Pb and 0.090 pc ^{210}Po. Student's *t*-test shows the mean values in the smokers to be significantly higher than in the nonsmokers ($P < .005$).

In smokers the ^{210}Po is nearly in radioactive equilibrium with its parent (^{210}Po : ^{210}Pb, $0.87 \pm .10$); that the two are closely related is shown by the correlation coefficient of 0.83 ($P < .005$). In nonsmokers a ratio of $0.62 \pm .14$ exists, suggesting a deficiency in content of the daughter; the *t*-test, however, shows the two means to be not quite significantly different ($P < .10$); the correlation coefficient of 0.61 is also low.

The previously reported (*7*) concentration of ^{210}Pb in trabecular bone (mainly rib and vertebra), 0.184 ± 0.018 (S.E.) pc per gram of ash, is significantly lower than that in smokers ($P < .005$) and probably significantly higher than that in nonsmokers ($P < .05$). These differences suggest that the previous sampling of the population was of a mixture of smokers and nonsmokers.