Severe Impairment of Heat-Induced Saliva-Spreading in Rats Recovered from Lateral Hypothalamic Lesions

Abstract. *A method has been devised to record saliva-spreading on fur and skin by rats in the heat. Normal males and females increase saliva-spreading with increasing ambient temperatures. Rats recovered from lateral hypothalamic lesions do not spread saliva in the heat, confirming the inference drawn from studies of their drinking behavior that their saliva production is severely impaired. Recovered lateral rats do produce saliva when injected with pilocarpine. The failure to salivate is, therefore, a result of the lateral hypothalamic lesions and is not due to an incompetence of the glands.*

Rats that have recovered from the initial aphagia and adipsia produced by lateral hypothalamic brain damage (recovered lateral rats) drink water only when they eat dry food (*1*). They have severe impairments in drinking to regulate body water. They do not drink in response to the dehydrating effects of hypertonic saline injections, water deprivation, or hyperthermia, and they do not drink water when they are not eating (*2*). They do drink while eating dry food. This style of drinking has been called "prandial drinking" or drinking at mealtime (*1*). It has been observed in neurologically normal rats without saliva (*3, 4*) (desalivate) as well as in the recovered lateral rat. The similarity in drinking pattern between desalivate and recovered lateral rats suggested that the recovered lateral has a chronic deficiency in the production of saliva (*5*).

Mammals produce saliva in the heat as well as during meals. Excess production of saliva at high ambient temperatures has been described for many species (*6*), and several of them utilize the saliva for evaporative cooling by spreading it on their fur and skin (*7*). The present report describes a technique for recording saliva-spreading in the heat. It demonstrates that the heat-induced salivary response is severely impaired in the recovered lateral rat, confirming the inference, drawn from studies of drinking patterns, that lateral hypothalamic damage impairs saliva production. Evidence is also presented concerning the nature of the heat-induced salivary response in normal animals. A sexual difference is described, and possible implications of saliva-spreading for temperature regulation are discussed.

The method takes advantage of the alkalinity of saliva to record its production in the heat. The floor of the animal's cage is converted into an alkaline indicator by covering it with 10- by 20-inch (25½- by 51-cm) sheets of bibulous filter paper permeated with cresol-red *p*H indicator solution (*p*H 7.2 to 8.8). The acidic paper titrates the indicator into its acidic, yellow range, and wherever the rat deposits saliva, it leaves a vivid reddish-purple spot against the yellow background. We call these records "spit-prints." Urine and sweat are acidic and, therefore, give no color reaction. This method does not measure saliva production per se, since some saliva is undoubtedly swallowed, and saliva is spread on the sides of the cage where it cannot react with the indicator. The method does faithfully record the saliva deposited on the floor of the cage and it reliably indicates changes in saliva-spreading as a function of ambient temperature.

Saliva-spreading was studied in seven adult Sprague-Dawley male rats (weighing from 350 to 420 g), two Sprague-Dawley females (320 to 350 g), and ten Sherman females (284 to 320 g). Six of the males were normal, and one was desalivated by ligation of the parotid and submaxillary ducts (*4*). Five of the females were normal, and five were recovered from the initial aphagia and adipsia of the lateral hypothalamic syndrome (*1*). It should be recalled that the neurosurgery necessary for the production of the syndrome does not involve the salivary glands or their peripheral innervation.

Each animal was studied in a temperature-control chamber at temperatures of 28°, 32°, 36°, and 40°C for 30 minutes without food or water. The animals were tested only once a day and were given at least one full day between tests. Excessive urination caused a dilution of the spit-prints, and experiments were repeated whenever this occurred. When the animals were not in the temperature chamber, they were housed in individual cages at a room temperature of approximately 25°C, with free access to Purina pellets and water.

Because the spit-prints faded slowly over several hours, they were photographed on 35-mm film after each experiment to provide a permanent record. For quantitative analysis the slides were projected (×30) onto a grid (10 squares/inch), and the area covered by the prints was determined. A source of error is introduced by the fact that the animals backtracked over their spit-prints, producing some overlap.

The specificity of the method for the detection of saliva was confirmed in two ways. First, no spit-prints were produced by the desalivate male at any of the four ambient temperatures used. Second, in five normal males atropine sulfate (0.28 mg) given intraperitoneally immediately before exposure to heat rendered the spit-prints recorded at 28° and 40°C indistinguishable.

There are two important effects of increased ambient temperature on saliva-spreading in normal rats. First, in both males and females saliva-spreading increases with increasing temperature. Second, there is a marked sexual difference in the extent of saliva-spreading in the heat. Both of these effects are shown in Fig. 1, where the area measurements of the spit-prints are presented for a representative animal from each experimental group. The animals in each group showed a distinct and uni-

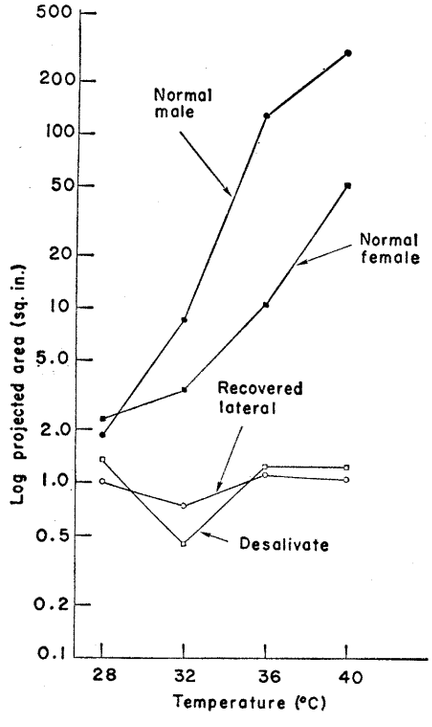

Fig. 1. Projected area of the spit-prints as a function of ambient temperature for a normal male, a normal female, a desalivate male, and a recovered lateral rat.

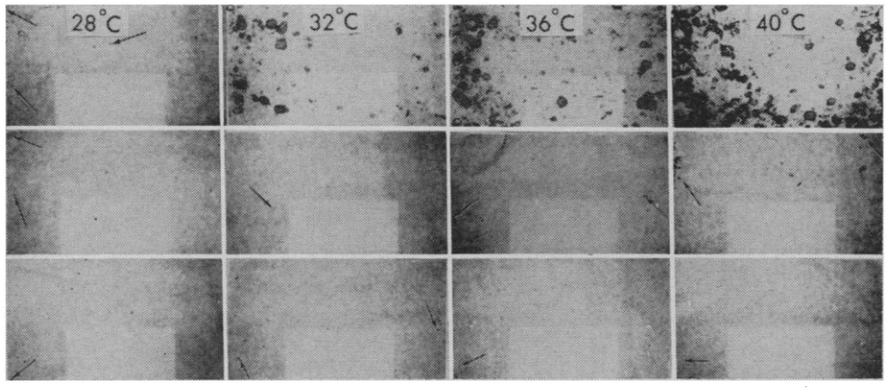

Fig. 2. Spit-prints made by a normal male (top row), desalivate male (middle row), and a recovered lateral rat (bottom row) at various ambient temperatures. The arrows point to the few prints made by the desalivate rat and the recovered lateral rat and to those made by the normal rat at the lowest temperature. The clear rectangles at the base of each print are photographic artifacts caused by identification cards which were photographed with each print and should be ignored.

form response that is represented by the animals selected for Fig. 1. In normal male rats, saliva-spreading begins at 32°C and continues to increase dramatically with higher temperatures. The spreading of saliva is associated with bouts of restless activity interspersed between periods of inactivity and prostration. When the male rat spreads saliva it grooms the vascularized surface of its head, feet, scrotum, and the base of its tail. At the higher ambient temperatures saliva often dripped from the chin during the periods of prostration and formed large prints on the indicator paper. Normal female rats of both the Sherman and Sprague-Dawley strain do not appreciably increase their saliva-spreading until the ambient temperature is increased to 36°C, and the area of the spit-prints for the females remains considerably less than the area for the males at the higher ambient temperatures. The normal females were also less active than the males in the heat, and they did not prostrate themselves as frequently.

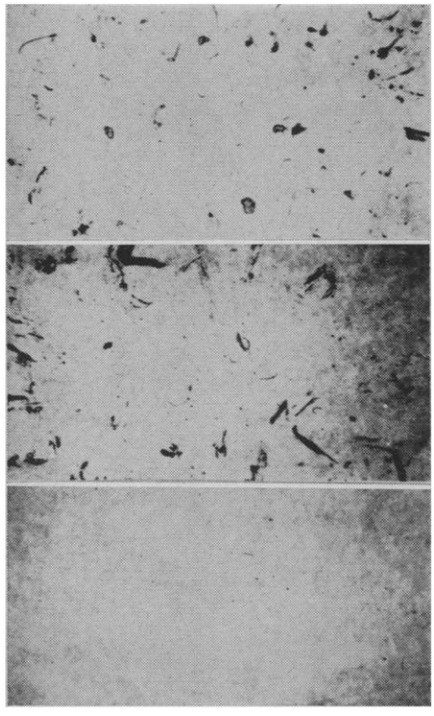

Fig. 3. Spit-prints made by a normal male (top), a recovered lateral rat (middle), and a desalivate rat (bottom) after pilocarpine injections at room temperature.

All five females that had recovered from the aphagia and adipsia of lateral hypothalamic lesions showed a severe impairment in the production of saliva in the heat. There was a slight increase in the spit-prints for one recovered lateral rat at 40°C and a complete absence of any temperature effect in the remaining four animals. The area measurements of the spit-prints for a representative animal are shown in Fig. 1. In contrast to the response of the normal female, note the lack of a temperature-dependent increase in the area measurements for the recovered lateral rat and the similarity of the recovered lateral rat's curve to that of the desalivate animal. Figure 2 presents representative spit-prints for a normal male, a desalivate, and a recovered lateral rat. The recovered lateral and desalivate rats produced very few prints (indicated by arrows) at each temperature, and because most of them were formed when the rats sniffed the paper, they appear to be the result of mucous secretions from the nose.

The recovered laterals were as active in the heat as normal females, and they made grooming and licking movements, as if attempting to spread saliva. The absence of spit-prints in these animals cannot, therefore, be attributed to a failure to perform the spreading movements. The deficit in the recovered lateral rat could be due to one or both of two additional possibilities: the salivary glands themselves could be deficient in function, or the neural control of secretion could be impaired. To distinguish between these alternatives, spit-prints were recorded for three of the normal males, the desalivate male, and the five recovered lateral rats after they had been injected with pilocarpine nitrate (2 mg, intraperitoneally) at room temperature. The results for a representative animal in each group are shown in Fig. 3. In every case pilocarpine elicited excess saliva production in both the normal and recovered lateral rats. The larger prints were made by the drooled saliva which pooled around their chins. From this evidence we conclude that the deficit in saliva production seen in the recovered lateral rat is caused not by an incompetence of the glands but by a failure of the neural control of saliva production in the heat. These rats do not salivate in the heat because the lateral hypothalamic lesions have destroyed brain tissue essential for the elicitation of the secretion at high ambient temperatures.

These results confirm the inference, drawn from studies of the drinking behavior of the recovered lateral rat, that saliva production is severely impaired in rats with lateral hypothalamic damage. They are prandial drinkers (*2*). That is, they drink water while they eat, taking a small draught of water immediately after a morsel of dry food is taken into the mouth. Rats without salivary glands also drink prandially when eating dry food (*3*, *4*), and Kissileff and Epstein (*5*) have recently shown that this style of drinking is produced by dryness of the mouth. It is unaffected by large intragastric water loads but is abolished by small injections of water into the mouth while the animals are feeding. The rat with lateral hypothalamic lesions, therefore, does not salivate normally when it eats dry food or when it is in the heat.

Finally, it has previously been assumed that rats and other small rodents lack an efficient means for heat loss by evaporative cooling since they do

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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References and Notes

1. P. Teitelbaum and A. N. Epstein, *Psychol. Rev.* **69**, 74 (1962).
2. A. N. Epstein and P. Teitelbaum, *Thirst*, M. J. Wayner, Ed. (Pergamon, New York, 1964), p. 395.
3. A. N. Epstein, D. Spector, A. Samman, C. Goldblum, *Nature* **201**, 1342 (1964).
4. W. B. Vance, *Psychol. Monog.* **79** (5), 1 (1965).
5. H. R. Kissileff and A. N. Epstein, *Federation Proc.* **24** (2) 523 (1965).
6. K. Robinson and D. H. K. Lee, *Proc. Roy. Soc. Queensland* **53**, 145 (1941).
7. A. C. Higginbotham and W. E. Koon, *J. Physiol.* **181**, 69 (1955).
8. J. W. Hudson, *Univ. Calif. Berkeley Publ. Zool.* **64**, 1 (1962).
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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,

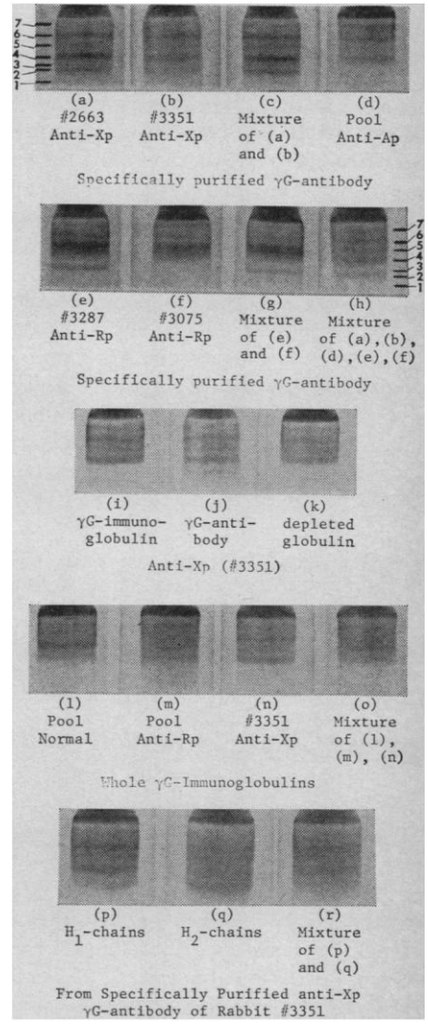

Fig. 1. Disc electrophoresis pattern (*p*H 4.3) of H-chains from rabbit γG-immunoglobulins.