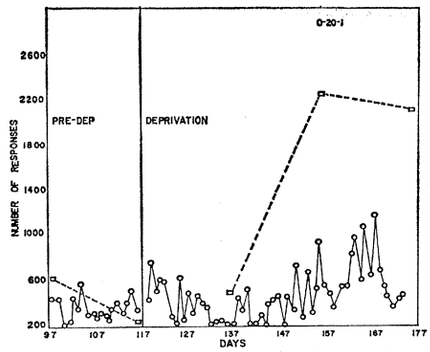

Fig. 1. Responses per hour of an animal in the 20-day group. The data to the left of the perpendicular line are for the predeprivation period; the data to the right, for the deprivation period. The circles connected by a solid line represent responding on the water-reinforced days; the squares connected by a dashed line trace the NaCl-reinforced behavior.

a reinforcement and the deprivation sessions in which the reinforcements were water or NaCl for the experimental groups and water or KI for the control group. The scores are expressed as ratios, as explained in the heading of Table 1. This ratio compensates for the different response levels of the rats during the predeprivation sessions.

These data show that near the end of the predeprivation period the hourly response rates of all of the subjects during the water-reinforced operant sessions were rather similar to their response rates during the sessions reinforced by a salt. At the end of the deprivation period, however, the response rate during the NaCl-reinforced sessions was greater than the response rate during water-reinforced sessions of the predeprivation period. This increase was less for the subjects in the 5-day group than for those in the 10- and 20-day groups. In fact, subject 1 of the 5-day group did not show this change. The responses of rats in the 10- and 20-day groups were roughly the same.

The use of potassium iodide as a reinforcement did not appreciably alter the behavior of the subjects in the control group. During the predeprivation period the KI-reinforced behavior of these animals was similar to their water-reinforced behavior.

Figure 1 shows the data obtained on a typical animal in the 20-day group. It should be noted that the increased NaCl-reinforced responding occurred after the 40th day of deprivation and that this increased rate is unique to those days on which NaCl was used as a reinforcement.

The increased responding during the NaCl test sessions seems to be specific to NaCl reinforcement, since only small changes occurred in the water- or KI-reinforced behavior. The change in the NaCl-reinforced behavior that occurred during the deprivation period can be related to Lewis's data (*3*). She found that a group of normal water- and salt-satiated rats would not respond in an operant conditioning apparatus when a 1-percent NaCl and water solution was used as reinforcement. A group of adrenalectomized rats, however, learned to press a lever under identical conditions. Thus NaCl deprivation appears to be necessary in order for NaCl to act as a reinforcing agent for a bar-press response.

The KI control was used to investigate the effects of NaCl deprivation in a situation in which the reinforcement contains ions that were adequately provided in the NaCl-deficient diet. The animals in this group reacted to the deprivation regime in the same fashion as their experimental counterparts: all subjects lost about 5 percent of their predeprivation body weight during the deprivation period. The control animals, however, did not show increased responding during the KI-reinforcement test sessions. This indicates that the increased response rate, which was characteristic of the animals deprived of and reinforced with NaCl, was not due to this weight loss. It is, of course, possible that the deprived animals would react differently if another salt solution were used as a control reinforcement. In this case the use of KI reinforcements did not change the behavior of the animals during the predeprivation period, and thus seems to produce a situation that was similar to the predeprivation NaCl test sessions.

During the deprivation period of the experiment the animals had access to two sources of NaCl: (i) that contained in the standard diet consisting of 4×10^{-4} percent NaCl, and (ii) that contained in the reinforcements provided during the NaCl test sessions (0.25 percent NaCl). Since the deprived animals ingested around 20 g of food each day, they consumed approximately 8.0×10^{-5} g of NaCl if we assume that all of the sodium appeared as NaCl. Sixty NaCl reinforcements contain 4.5×10^{-3} g of NaCl. Since all of the animals received the same food during deprivation, the only difference between the groups is the amount of NaCl they received during the test sessions. The 5-day group had one more salt test period than the 10-day group every 10 days. This gave them an average advantage of 4.5×10^{-4} g of NaCl per day. Despite the small differences in NaCl intake among these groups, the 5-day animals failed to show the deprivation effects as severely as the 10-day animals. These differences indicate that very small changes in the amount of NaCl deprivation can alter the subject's behavior.

The results of the present experiment clearly indicate that the effects of NaCl deprivation can be studied through the use of operant conditioning techniques. The sensitivity of this measure also indicates that the technique may be useful in the analysis of the dietary needs of organisms.

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25 April 1963

Cardiovascular Responses of the Chicken to Seasonal and Induced Temperature Changes

Abstract. *Blood pressure and cardiac output decline as ambient temperature rises in birds acclimatized to both seasonal- and induced-temperature changes, in contrast to the response usually observed in unacclimatized mammals. The decline in chickens is due to a lowered vascular resistance and blood volume. These circulatory adjustments may be related to the fact that excess heat in birds is dissipated through the respiratory system rather than through the skin.*

Most studies of circulatory responses to changes in ambient temperature have been short-term experiments of a few hours or a few days which is insufficient time for long-term adaptative processes to develop such as may occur under seasonal influences.

Weiss, Ringer, and Sturkie (*1*) first observed a seasonal change in blood pressure of chickens (higher levels in the winter), and further work substantiated their findings (*2*). Seasonal changes have also been studied in man (*3*) and other animals (*4*), but observations have been limited to blood pressure, blood volume, and heart rate.

Exposure to high temperature for short periods usually produces an initial increase in cardiac output (*5*) which later may return to normal. Heart rate, blood pressure, and blood volume generally increase—this increase depends on how high and how quickly the temperature rises.

Our objectives were first to determine the seasonal effects on cardiac output and peripheral resistance in chickens adapted to summer and winter temperatures, and second to determine the effects of induced temperature differences on these parameters since the possibility exists that factors other than temperature may result in seasonal circulatory changes. An attempt has also been made to elucidate the specific cardiovascular mechanisms in adaptation to ambient temperature.

Cardiac output and blood pressure were determined on male and female adult White Leghorns kept in small open-sided floor pens on the experimental farm and subjected to outdoor temperatures. Measurements were taken during February and August of the same year. Egg production was approximately the same during both months (70 percent) and no evidence of molt was found. Artificial light was used to produce a uniform 15-hour day. The birds were brought from their pens to the laboratory (70°F) and held no longer than 1 hour before the determinations were made. Mean maximum and minimum temperatures for February were 40° and 26°F and for August 81° and 64°F.

In a second experiment, cardiovascular responses to ambient temperatures of 30°, 70°, and 95°F were studied in hens. The hens were initially divided into two groups: one remained in open outdoor pens and the other was placed in cages in the laboratory where room temperature was first raised to 85°F for two weeks and then to 95°F, and then maintained at this temperature for 17 days before cardiac output and other measurements were made; relative humidity was maintained at 60 percent. After determinations had been completed on the outdoor birds (February temperature), they were subjected to a temperature of 70°F for 2 weeks and measurements were again repeated which gave the third temperature comparison.

The birds were restrained but not anesthetized with little resultant excitement to the animal. Blood pressure was measured directly from a common carotid artery with a Statham transducer and Sanborn recorder, and cardiac output by the Hamilton dye-dilution technique adapted for the chicken (*6*), except that a Waters continuous-flow densitometer and indocyanine green dye were utilized. Four milliliters of blood were necessary for a determination. Tracings of the curves were made on a Rectiriter galvanometer recorder with three determinations possible within a 10-minute period. Total peripheral resistance was calculated in arbitrary units by dividing mean blood pressure (millimeters of mercury) by cardiac output (milliliters per minute).

Table 1. Effect of season on the circulatory system of chickens. Values are means and standard error.

Measurement	Male		Female	
	February	August	February	August
No. of birds	20	16	20	19
Body wt (kg)	2.59 ± .07	2.95 ± .09*	1.95 ± .04	1.96 ± .06
Mean blood pressure (mm-Hg)	181 ± 4.7	177 ± 4.8	153 ± 3.5	147 ± 2.7
Heart rate (per min)	303 ± 8.2	289 ± 10	336 ± 24	347 ± 9
Cardiac output (ml/min)	444 ± 22	359 ± 11*	345 ± 15	234 ± 7*
(ml/min kg)	173 ± 9	135 ± 7*	181 ± 12	121 ± 5*
Stroke volume (ml)	1.50 ± .06	1.20 ± .06*	1.04 ± .06	0.68 ± .03*
Total peripheral resistance				
units	0.42 ± .02	0.53 ± .03*	0.46 ± .13	0.59 ± .02*
units/kg	1.11 ± .07	1.41 ± .10*	0.90 ± .13	1.25 ± .07*

* Difference between months significant at .05 level.

Measurements of cardiac output of both sexes were approximately 23 and 33 percent lower in summer than in winter (Table 1). Values for total peripheral resistance were 20 percent higher in summer. The reduced cardiac output was due to a lower stroke volume, for the heart rate did not change. The differences in blood pressure were not statistically significant but tended as usual to be higher in winter. The absence of a larger seasonal difference can be attributed partially to an aging factor, since the birds tested in summer were 6 months older. An increase of 5 to 6 mm-Hg in arterial pressure with age over the period, as reported by Weiss *et al.* (*2*), would tend to minimize the seasonal effect in our study.

Though temperature is the most striking change between two seasons, other factors, such as reproductive state and duration of natural daylight, could also account for circulatory adjustments. Rate of egg production during both periods was comparable and no molting was observed. Furthermore, no relationship has been found between reproductive state and blood pressure in the chicken (*7*). As further evidence that temperature is the causal factor, our records have shown that cyclic changes in blood pressure are generally not observed in chickens housed in battery rooms maintained at constant temperature.

Blood pressure measurements of chickens exposed to 30°F were significantly higher than those exposed to 95°F, but not significantly different from those exposed to 70°F (Table 2). Differences in heart rate were insignificant. Cardiac output per kilogram decreased with increasing ambient temperatures from 270 ml/min at 30°F to 163 ml/min at 95°F. The latter value was significantly different from that obtained at the lower temperatures. Total peripheral resistance was significantly higher for the birds at higher temperatures. A subsequent trial showed that blood volume dropped from 6.5 to 5.2 percent of body weight or a 20 percent change from 70° to

Table 2. Effect of induced high temperature on the circulatory system of female chickens. Values are means and standard error.

Measurement	30°F winter	70°F	95°F
No. of birds	12	6	6
Body wt (kg)	1.76 ± .08	1.69 ± .06	1.60 ± .06
Mean blood pressure (mm-Hg)	147 ± 3.6	144 ± 11.4	127 ± 2.2
Heart rate (per min)	346 ± 14.3	356 ± 10.3	322 ± 15.7
Cardiac output (ml/min)	481 ± 38	402 ± 12	262 ± 43*
(ml/min kg)	279 ± 11	240 ± 24	163 ± 25
Stroke volume (ml)	1.44 ± .70	1.12 ± .08	0.83 ± .14
Total peripheral resistance			
units	0.34 ± .04	0.37 ± .03	0.57 ± .10*
units/kg	0.59 ± .07	0.62 ± .16	0.89 ± .14*

* Difference between 95° group and other groups significant at .05 level.

95°F. Plasma volume declined 13 percent.

In the second study, housing conditions were different for birds subjected to 30°F and birds subjected to the two higher temperatures and therefore this might have been an additional factor. Blood pressure has been found to be higher in birds housed in floor pens than in those caged communally or individually (*2*). Physical activity rather than the presence of other birds may influence pressure. However, seasonal changes in blood pressure were prominent in all birds and therefore the type of housing did not seem to account for any major portion of the differences.

Our findings do not correspond to those reported for mammals, perhaps because the mechanisms bringing about circulatory adaptations to heat are different. The increase in cardiac output in man at high ambient temperatures presumably results from peripheral vasodilation and an increased vascular volume which in turn calls for a greater cardiac output to facilitate heat loss through the skin. In contrast, chickens loose heat primarily through the respiratory system instead of through the skin. Blood and plasma volume actually decreased with adaptation to heat; such a decrease would diminish venous return and lower cardiac output.

These results are from acclimatized birds and may not be comparable to most findings reported for mammals. Hyperthermia, which increases output, was not a condition in our experiments (*8*).

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28 March 1963

Antibody to Rat Kidney: In vivo Effects of Univalent and Divalent Fragments

Abstract. *Whereas intact antibody to rat kidney (7S) produced immediate and sustained proteinuria in rats, univalent fragments (papain digests) of the antibody did not, and divalent fragments (pepsin digests) produced only transitory proteinuria. The antibody fragments differed from the intact antibody in fixing little, if any, complement in vivo which may explain why they did not cause serious renal damage.*

We have compared in rats the effects of intact antibody to rat kidney and fragments of the antibody prepared by the methods of Porter (*1*) and Nisonoff (*2*). Antikidney serum produces renal damage; the damage in rats is manifested by proteinuria which is a very satisfactory measure of the severity and persistence of the renal lesion (*3*).

Antikidney serum from rabbits that had received repeated intraperitoneal injections of rat kidney stroma without adjuvants (*3*) was fractionated on diethylaminoethylcellulose (*4*). The effluent from treatment with 0.0175*M* phosphate at *p*H 6.3 contained only 7*S* γ-globulin. This fraction produced immediate and persistent proteinuria when injected intravenously into rats (Table 1). Material eluted in a subsequent fraction containing the macroglobulin also produced proteinuria. Further resolution of this subsequent fraction on a Sephadex G-200 column showed, however, that the nephrotoxic component was in the contaminating 7*S* material rather than the macroglobulin. When it was established that the nephrotoxic component was associated only with the 7*S* antibody, sodium sulfate (*5*) was used in the later studies to separate γ-globulin.

The 7*S* γ-globulin was digested with papain (*1*), and piece III was allowed to crystallize in the cold. The supernatant solution containing 3.5*S* univalent antibody fragments I and II produced neither immediate nor delayed proteinuria (Table 1). Two exceptions were noted wherein mild and evanescent proteinuria occurred during the fourth week after injections. Piece III alone and the univalent fragments plus piece III were likewise ineffective in producing proteinuria.

A pepsin digest (*2*) containing divalent (Nisonoff) 5*S* fragments of the antikidney γ-globulin produced transient proteinuria in rats—after the usual doses the proteinuria disappeared in 48 hours (Table 1). Even after the larger doses, proteinuria disappeared within 6 days. The 5*S* fragments purified by either sodium sulfate precipitation (*6*) or Sephadex G-100 gel filtration produced the same transient proteinuria, whereas the small fragments thus removed were ineffectual. The proteinuria was not attributable to residual undigested antibody in the injected material, for no significant amount of 7*S* material was present (Fig. 1). Furthermore, reduction of the digest material with β-mercaptoethanol (*7*), which does not inactivate the antibody if present, degraded the 5*S* fragments to 3.6*S*, and the material no longer produced proteinuria.

Fragments prepared from nonspecific γ-globulin were injected into control animals. Both the univalent and divalent nonspecific fragments in the usual doses (those obtained from 2 to 4 ml of serum) failed to produce significant proteinuria. In doses obtained from 10 to 20 ml of serum, however, both types of nonspecific fragments and also the specific univalent fragments induced mild proteinuria, averaging less than 2 mg of protein per hour and limited to the first day which apparently was the result of excretion of the injected material. This proteinuria was clearly less in intensity and duration than that caused by equivalent doses of the specific divalent fragment.

Antibody fragments, both univalent

Table 1. Proteinuria produced in rats by antibody to rat kidney and its fragments. Additional dosages were used and urine was collected periodically for at least a month (*3*). The figures in parentheses indicate the number of animals tested.

Dose* (ml)	Protein (mg/hr)		
	Day 1	Day 3	Day 21
Normal controls† (*12*)			
	0.2	0.3	0.1
Antiserum to rat kidney (*9*)			
1 to 2	9.4	8.8	3.7
7S γ-globulin (*7*)			
1 to 2	8.0	7.1	5.0
Univalent pieces I and II (*7*)			
2 to 4	0.3	0.3	0.2
Divalent 5S pieces (*6*)			
2 to 4	3.2	0.3	0.1
Divalent 5S pieces (*3*)			
5 to 10	6.8	1.3	0.2

* Dose in terms of antikidney serum (adjusted to standard potency) from which the material was derived. † Similar results were obtained with rats receiving no injection and rats injected with 1 to 2 ml of normal rabbit serum. No individual protein values above 0.5 mg/hr were observed in these control rats.