Technical Papers

Some Metabolic Responses of Dogs Having Low Body Temperature[1]

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The impression that valuable physiological information could be obtained from long-term studies conducted on homeothermic animals having a reduced body temperature has prompted a number of investigators to obtain such a preparation (*1–3*). Woodruff (*3*) concluded that dogs are unable to survive hypothermia sufficiently long to make protracted metabolic studies feasible. The longest period that he was able to maintain a dog with survival at a rectal temperature below 82° F (27.8° C) was 10½ hr. However, in one instance an animal was maintained at this level for 26 hr before succumbing. Similar experiments have been conducted in this laboratory during the past 2 years, and some success has been obtained in producing a preparation whose rectal temperature could be maintained at approximately 24 ± 1° C for periods up to 28 hr with survival.

The method employed, in brief, consisted of preliminary anesthetization of the dog with 30 mg/kg of Nembutal[2] to permit control of the initial shivering during cold exposure and the obtaining of control data for a 1-hr period in a control room having an ambient temperature of 26° C. The animal was then moved into another room where the temperature was approximately −10° C. The Nembutal generally inhibited the shivering usually observed, and the rectal temperature dropped rapidly to approximately 28° C, at which time the room temperature was raised to about 22° C, and by minor shifts of this environmental temperature, the dog's rectal temperature was stabilized around 24° C. Thereafter the animal remained very quiet, usually without exhibiting gross shivering movements until the room temperature was once again raised to initiate the rewarming processes. The animals recovered spontaneously and were fully awake and aware of events when their rectal temperature attained 28° C. One animal was studied in this manner on 5 occasions and apparently suffered no ill effects.

The neurological, chemical, cardiovascular, and other phenomena which were studied in these animals will be reported in detail in later publications. It is the intent of this report to call attention briefly to the metabolic activity and energy exchanges observed in two of the latest experiments. The heat production of these two animals was measured in one instance (animal B) by a direct oxygen consumption method, that of Benedict-Roth, and in the other (animal A) by analysis for CO_2 and O_2 in an aliquot of the expired air collected in a Tissot spirometer. Temperatures of the room air, walls, rectum, muscle, various skin areas, and blood were obtained at frequent intervals by means of suitable copper-constantan thermocouples and a recording Brown potentiometer.

Some of the pertinent data obtained in these two experiments are summarized in Table 1. Although the magnitude of the physiological alterations in these animals is readily evident, the remarkable constancy of the preparation can only be partially appreciated by the statement that the rectal temperature remained constant. Further evidence of this stability comes from the perusal of data obtained on heat production and heat loss. In animal B the heat production during a 35-hr period varied between 10.9 and 6.6 and averaged 9.2 Cal/M²/hr. Similarly, animal A had an average heat production of 11.3 Cal/M²/hr for the last 27 hr of the experiment. The reduction in heat production and the fall in rectal temperature observed in 18 experiments performed on 11 animals appeared to have an exponential relationship (Fig. 1). The line drawn

TABLE 1

OBSERVATIONS ON TWO ANIMALS WHOSE BODY TEMPERATURE WAS MAINTAINED AT A LEVEL APPROXIMATELY ONE-THIRD BELOW NORMAL

	Dog A (30 hr) open circuit		Dog B (38 hr) closed circuit	
Weight kg	19.0		24.3	
S.A. M²	0.80		0.99	
	Control	Low	Control	Low
Rectal temperature °C	36.7	23.6*	35.8	23.5†
Mean body temperature °C	35.8	22.7	34.5	22.5
Heart rate/min	84	36	114	44
Calculated heat content Cal/M²	705	448	690	451
Oxygen consumption ml/min	96	17	129	23
Heat production Cal/M²/hr	34.7	6.2	37.7	6.6
Respiratory rate, per minute	8	2	6	1
Respiratory minute volume L/min	2.0	0.2	2.5	0.5
Total heat loss Cal/M²	219		239	
Total heat production Cal/M²	436		416	
Theoretical total heat production Cal/M²	1048		1433	

* 28 hr at a rectal temperature within 1.5° C of this value. This animal was later used in other experiments.

† 37 hr at a rectal temperature within 1° C of this value. This animal died while being rewarmed.

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[2] Owing to the lowered metabolic activity observed in these animals in the cold, the possibility that continued Nembutal activity rather than cold per se is maintaining these animals has been considered. Several other shorter-acting anesthetic agents have been employed, and the relationship of initial anesthesia and the cold stimulus will be considered in detail in a future publication.

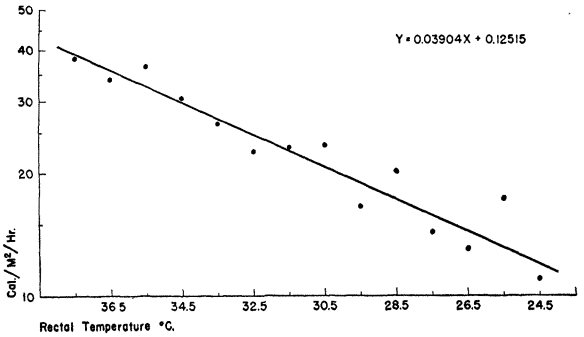

FIG. 1. Relationship between rectal temperature and heat production observed in 18 experiments on 11 animals.

through the observed data was fitted by the method of least squares. Application of van't Hoff's law to the warm-blooded organism within this temperature range would appear to be valid. Whether this observation can be extended to energy exchanges in separate organ systems cannot be answered at the moment, but studies are now in progress to elucidate this point.

Further and more comprehensive reports of the studies being conducted on animals maintained at this reduced and stable body temperature will appear shortly.

References

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Chemical Transformation of Steroids by Adrenal Perfusion VI. Allopregnane-21-ol-3,20-dione[1, 2]

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A multicycle perfusion of allopregnane-21-ol-3,17-dione (I) through isolated cow adrenals was performed a year and a half ago. In the course of working up the perfusate, difficulties in purifying the transformation products were encountered. Therefore a repetition of this perfusion was planned applying the newly developed processing techniques (*1*). Since, however, some time will elapse until this work can be started, and in view of a recent communication in which one of the transformation products of the above perfusion (allopregnanediolone) was described as a rat liver metabolite of desoxycorticosterone (*2*), it was felt that a preliminary report was advisable.

[1] This investigation was supported by a grant from G. D. Searle and Company, Chicago, Illinois.

[2] The acetoxyallopregnandione was donated by A. White, Chemical Specialties Company, Inc. Thanks are due to L. Ruzicka and H. Heusser for their generous cooperation. Infrared analyses and interpretations were kindly made by H. Rosenkrantz.

Purified I (m.p. 158–165°, $[\alpha]_D^{22} + 104.8 \pm 1.5°$ in chloroform) was readily converted by the surviving adrenals. Relatively little starting material was recovered. At least six transformation products at levels exceeding 1% were distinguished by their elutability in the silica gel chromatography. Of these, the following two have been identified.

The 11β-hydroxylated product, *allopregnane-11β, 21-diol-3,20-dione* (II), was found in relatively low concentration compared to the quantities of corticosterone formed in corresponding Δ^4-unsaturated desoxycorticosterone perfusions (*3*). Other 11-hydroxylated compounds might possibly be present, since several fractions were not identified. Hayano and Dorfman subsequently found with their beef adrenal residue preparation that I was converted to the 11β-hydroxylated derivative only approximately one half as readily as was desoxycorticosterone (*4*). The identification of compound II was completed as follows. An authentic sample of allopregnane-11β,21-diol-3,20-dione (*4*) was converted into the 21-monoacetate and found to be identical with the acetate of II (m.p. 189–192°) by mixed m.p. and infrared spectrometry. Absorption bands of a solution in carbon disulfide were found near 3460 (hydroxyl), 1751, and 1231 (acetate), 1730 (carbonyl at C.20), 1715 (carbonyl at C.3), 1087, 1054, and 1040 cm^{-1} (some fingerprint bands).

Another transformation product, obtained at a 15% level, was established as the reduced derivative *allopregnane-3α,21-diol-20-one* (III) as follows. After several crystallizations from ether, the m.p. 156–162° was attained. Admixed with compound I, a m.p. depression of 40° was observed; III showed the reducing properties of a steroid with a 20,21-ketol side chain toward silver ammonio reagent and was not precipitable with digitonin. Since the crystals appeared to be not entirely pure, the substance was converted to the acetate and yielded analytically pure leaflets with m.p. 165–166.5°, $[\alpha]_D^{27} + 98 \pm 2°$ (*c*, 0.710 in chloroform).

Analysis	$C_{25}H_{38}O_5$	Calcd.	C	71.75	H	9.15
		Found	C	71.66	H	9.21

These data suggested that this product was identical with allopregnane-3α,21-diol-20-one-diacetate (m.p. 165°, $[\alpha]_D + 92°$ in chloroform) (*5*). Ruzicka and Heusser kindly made a comparison of the acetate of compound III with their authentic material; the admixture did not produce an m.p. depression. Upon receipt of a sample of the authentic material, the identity of the substances could be substantiated by infrared spectrometry. Absorption bands of both samples in the solid state were found near 1739 and 1237 (ester groups), 1073, 1052, 1038, and 1019 cm^{-1} (some fingerprint bands). The keto group at C. 20 was only weakly resolved, visible by an inflection at 1710 cm^{-1}.

The formation of the corresponding 3β-hydroxyl derivative can for the moment not be excluded since several substances were not identified. In any case, its