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REPORT: Annual Technical Status Report.

SUBJECT: The Effect of environmental temperatures on the activities of certain enzymes in mammalian skin.

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J.N.R.GRAINGER.

b. ABSTRACT

A satisfactory method is described for maintaining one ear of a rabbit at about 30°C while the opposite ear is at about 12°C. The animal is kept in special stocks during the experiment. It was found necessary to remove the hair from the ears 1-2 days before the experiments. Ear hair growth is much more rapid in rabbits kept at 24°C than in rabbits kept at 6°C. This is probably due to the difference in ear temperature in the two cases. Rabbits kept at 6°C air temperature had ear skin temperatures ranging from an average of 16.2°C at the ear base to 12.19°C at the ear tip. Rabbits at 24.5°C air temperature had ear temperatures ranging from a mean of 34.7° at the base to 35.1°C at the tip. In all the above measurements (as with all the experiments reported in this paper) the hairs had been removed from the ear using a depilatory compound some days beforehand.

There is a small but significant (P = 0.05) difference between the rates of oxygen consumption of minced ear skin, when measured at 30°C, from cold and warm adapted rabbits. The respective means were 1.92 and 1.64 ul./mg N/ hour.

Succinic dehydrogenase is present in reasonable quantities in the ear skin of the rabbit. The activity of this enzyme when measured under standard conditions is very much higher in skin from cold-adapted rabbits ears as compared with warm adapted ear skin. Furthermore, this very significant difference becomes established within the short time of two days at 6°C. The respective means of the activity measurements are as follows: Cold-adapted (3 weeks at 6°C) 0.466, warm-adapted (3 weeks at 24°C) 0.319, warm-adapted and then 2 days at 6°C 0.442 ug. 2,3,5 triphenyl tetrazolium chloride reduced / mg wet weight of tissue/hour at 37°C.

Arginase is present in rabbit ear skin but in such small quantities that its measurement is not satisfactory. Attempts to make accurate measurements on this enzyme were later abandoned.

c. REPORT

All the experiments were carried out on white rabbits obtained from a dealer. These were kept in wooden cages at first with straw on the bottom but later these were changed to specially constructed wire cages as the wooden cages provided too much insulation when the rabbits were exposed to the cold. The rabbits were fed on a standard diet. Two main adaptation temperatures were used; a warm constant temperature room at 24.5°C (this was later altered to 20°C) and a cold room at 6°C. In both the air was circulated by fans.

Ear temperatures were recorded using a thermistor thermometer. The thermistor was a F23 Stantel thermistor and it was used in a bridge circuit with a variable arm and a sensitive null point detector across the bridge. The values of the resistors were calculated by the method given by Moore and Mortimer (1953) so as to give the maximum sensitivity over the temperature range used. Two ranges were employed 0-20°C and 20-40°C. Skin temperatures could with this instrument be measured to 1/20th of a degree.

In all the experiments reported in this paper the hairs on the rabbits ears were first removed with a depilatory agent 'Veet' and were then well washed. This was done 1-2 days prior to the experiment. This method proved very satisfactory and appeared to have no ill effects on the skin if washed off well. The hairs at the very edges of the ears were not removed by this treatment. It was noticed that the rabbits kept in the warm room after this treatment showed a much more rapid growth of hair on the ears than those kept in the cold room where hair growth seemed to be very slow on the ears.

This may be due to the fact that the skin temperatures (and hence the hair follicles) were much lower in the cold room rabbits. This is shown by the following table.

SKIN TEMPERATURES OF RABBIT'S EARS

Air temp.	6°C (wire cages)	6°C (wooden cages)	20°C	24.5°C
Ear base front	16.25	17.40	34.18	34.76
middle front	12.69	15.55	31.99	34.43
tip front	12.19	17.67	34.86	35.10
side front	13.00	16.71	30.94	35.01
mid back	13.52	16.51	31.57	35.30
Head of rabbit	35.20	35.25	-	36.28
Back of rabbit	32.22	32.68	35.26	35.59
No. of ears measured	10	16	4	12

It will be seen from this table that the wooden cages used had an appreciable insulating effect in the cold. All the later experiments (which are the ones reported primarily in this paper) were carried out in wire cages. It will also be noted that in the cold the temperature falls towards the tip of the ear, there being about a 4° difference in the wire cages rabbits. In the case of the warm room rabbits there was little difference between the tip and the base of the ear. The measurements on the head and the back were taken under the fur and of course show the insulating effect of this. From the above table one can say that the difference in ear skin temperatures between the 6° rabbits (wire cages) and the 24.5° rabbits was approximately 22°C.

A method has been developed for maintaining a temperature differential between the two ears of an individual rabbit. Although no experiments have yet been carried out using this method it will be described so as to avoid describing it in later reports. The rabbit is first trained to sit in stocks. The conventional stocks are not satisfactory as eventually the rabbit kicks with its hind legs and does itself serious injury and usually has to be destroyed. It was found to be essential for the rabbits to be able to live for several days in the stocks. A box well padded with spongy rubber with numerous holes for air circulation was constructed through which the rabbit's head protruded. The sponge rubber is essential because otherwise the rabbit will eventually kick and break its back. It is necessary to subject the rabbit to a course of training lasting several days to get them used to the box before the experiment. When this has been completed the rabbit is placed in the box with a supply of food and water available. A narrow can with a vertical partition soldered up it was now placed over one ear. Some cotton wool was placed between the wall of the can and the ear. A 15 Watt electric bulb was located in the other half of the can. This bulb was separated from the ear by an air space and the metal partition. Where the can touched the head of the rabbit it was well padded. It was placed in such a position that the rabbit could make a limited amount of movement of its head. The whole apparatus was now placed in the 6° room and it was found that after a short time there was a differential of nearly 20° between the two ears. The rabbit once it got used to the can could remain like this for several days. Detailed results using this method will be presented in later reports.

The experiments reported below were carried out on 'warm adapted' and 'cold adapted' rabbits. In view of the finding by Heroux (1959) that mitotic activity in the ears of rats kept at 6° is almost completely blocked for the first 3 weeks and then recovers, it was thought advisable to keep the present rabbits for at least 3 weeks at the adaptation temperatures before carrying out experiments on them. The warm adapted ones were kept in a constant temperature room at 24.5° (in the oxygen consumption experiments) and the cold-adapted ones in a constant temperature room at 6°C. The

rabbits in the oxygen consumption experiments were kept in wooden boxes so the difference in ear temperatures between the 6° and 24.5° rabbits was somewhat less than in the succinic dehydrogenase experiments which will be described later.

A number of experiments were carried out to determine whether there was a difference in the oxygen consumption of minced skin from cold and warm adapted rabbits. the rabbits were killed by a blow on the head. The ears were taken off and the skin was stripped off the central piece of fibrous tissue of the ear. It was cut into small pieces with a scissors, a portion of tissue being kept for a later nitrogen determination. The chopped skin was then scraped with a sharp scalpel through a guard of a Ronson electric razor. This method, described in Grainger (1960), proved very satisfactory and 'diced' the skin into small pieces about 0.5 mm square. Weighed amounts of minced tissue were placed in Warburg flasks containing 2 ml. Krebs-Ringer Phosphate (pH 7.4), 1 ml. 0.1 M glucose. The centre well contained 0.2 ml. 3M KOH. Experiments were carried out at 30.0°C. The means for each rabbit are given in the following table.

O_2 consumption (ul./mg.N/hour)

6° adapted	24.5° adapted
1.721	1.458
1.774	1.803
2.104	1.381
2.130	1.618
1.680	1.869

3-4 readings were normally made on each rabbit. The overall means were 6°- 1.924, 24.5°- 1.641. A statistical analysis gave t = 2.383 for 8 degrees of freedom (P = 0.05). There was no significant difference between the variances of the two groups ~~of values~~ (F value). Thus one can say that the skin from the cold adapted rabbits shows a significantly higher rate of oxygen consumption than the skin from the warm adapted ones although the difference is not a dramatic one.

In view of the difference in oxygen consumption described above it was decided to investigate succinic dehydrogenase activity. In these experiments the warm-adapted rabbits were kept at 20.0°C. and the cold adapted at 6°C. All were kept in wire cages. The activity of succinic dehydrogenase was measured on thin strips of skin using the method described by Kun & Abood (1949) as modified by Desmarias (1955). Only skin from the front of the rabbits ears was used. Some measurements were made on skin from the back of the ear and these indicate that the activity of this enzyme is lower there. Some strips were kept for nitrogen determination. All the nitrogen determinations are not yet available so the results given below are given in terms of wet weight of tissue.

Succinic Dehydrogenase Activity (ug. triphenyl tetrazolium reduced/mg wet wt. tissue/hour at 37°)

6° adapted	20° adapted	20° adapted and then 2 days at 6°C.
0.466	0.261	
0.589	0.309	0.455
0.411	0.303	0.540
0.361	0.394	0.361
0.454	0.387	0.411
0.491	0.292	
0.484	0.310	
0.544	0.298	
0.536		
0.363		

Overall means 0.466, 0.319, 0.422

The figures in the table are the means for each rabbit. Normally 8-10 estimations were made on each rabbit. The overall means are calculated on the individual estimations. A statistical treatment of the results gave the following results. There is a highly significant difference between the means of the cold and warm adapted rabbits ($t=4.77$ for 16 degrees of freedom, $P<0.01$). There is no significant difference between the cold adapted group and the warm adapted which had been at 6° for 2 days ($t=0.556$ for 12 degrees of freedom, $P>0.5$). There is a very significant difference between the warm adapted and the warm adapted which had then been placed for 2 days in the cold ($t=3.196$ for 10 degrees of freedom, $P<0.01$). Thus it appears that the increase of about 46% in succinic dehydrogenase activity between the warm and cold adapted groups largely takes place during the first two days at 6°C. The number of rabbits in the 2-day group is low. It is planned to do some more experiments in this group. These results are similar to those obtained by Desmarias (1955) on the abdomen skin of rats. The succinic dehydrogenase results fit in well with the oxygen consumption results in which there is also an increase in the cold, although it should be borne in mind that there is not necessarily a connection between the rise in both.

Some experiments were carried out on arginase activity since it had been reported that this enzyme was confirmed to the epidermis (Mardashev & Semina, 1948). A method was developed based on Roberts (1948) except that the urea formed was determined using urease and trapping the ammonia produced in standard acid placed in the centre wells of Conway units and then backtitrating. This showed that the arginase activity was extremely low. At 38°C the activity was 1.7 - 4.4 ug. arginine attacked/mg wet weight tissue/hour. In view of this further work on arginase abandoned.

The nitrogen estimations in all the experiments were done by digesting a weighed amount of skin in Kjeldahl flasks in the usual way and then following dilution the ammonia was measured by Nessler's solution in a colourimeter.

Implications of research described above:

The first objective of the original contract has now been attained, namely that a concrete difference has been established between the skin of cold and warm-adapted rabbits. The next stage is see whether this can be brought about in one individual in which one ear is cooled and the other warmed. This is the first step in deciding whether the differences described above are central effects brought about by neural or humoral means or whether they are purely local effects due to the tissue itself being at different temperatures in the two cases. The method for carrying out this experiment is described above and that is the next objective of the work.

The increase in the oxygen consumption of isolated skin in cold adapted animals is very similar to the changes which have been observed in many cold-blooded animals. In fact in this respect the rabbit ear is behaving rather like a tissue from a cold-blooded animal. Thus it is not surprising to find that changes in activity of some enzymes (e.g. succinic dehydrogenase) take place with changes in adaptation temperature. Similar changes are well known in cold-blooded animals (Precht et al., 1955). Other authors (e.g. Hannon, 1960) have reported increases in succinic dehydrogenase activity in various organs other than skin following exposure of rats and mice to the cold. This might suggest that there is some general humoral effect causing this. However future experiments may clarify the situation.

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ANNEX

1. PERSONNEL:

From 1st April, 1962, to 30th September, 1962, Mrs. P.M.C.Davies, B.Sc., was my assistant. She then resigned due to marriage commitments and was replaced by Miss C. Joyce, B.Sc., who started work on 1st October, 1962, and is still with me.

RESEARCH:

There has been no major change in research policy since the contract started and none is now envisaged. The first basic object has been to establish whether a difference was to be found in the activities of an enzyme in the ear skin of warm adapted as compared with cold adapted rabbits. Such a difference was found and has been thoroughly established in the case of succinic dehydrogenase. This work has necessarily been slow owing to the necessity for keeping the rabbits long enough in the cold to ensure that they were without any doubt fully adapted to the cold. It was decided that a minimum period of 3 weeks was necessary for this. Because of the very limited cold room accommodation this slowed down the work very much. This problem has now been largely overcome by increased cold room accommodation and the fact that we now know that it is not necessary to keep the rabbits for such a long period in the cold for a marked change in succinic dehydrogenase to take place. Another factor which retarded the work at the beginning was that hair on the ears was not removed before exposing the rabbit to the cold or warm environment, with the result that there was a much smaller difference in ear skin temperature between the cold and warm groups than there has been in recent experiments. In view of this many of the earliest experiments were discarded.

2. MANHOURS AND COST:

Manhours worked during the year 1st April, 1962, to 31st March, 1963, were:

Principal investigator	360
Assistant	1980
	2340 manhours.

The main expense in materials has been on rabbits. The cost of these is increasing but the contract should be enough to cover this during the current year since the expenditure on chemicals and glassware will be less this year. A cost breakdown of the year's expenditure (excluding salaries) is:-

Rabbits	72
Glassware	125
Chemicals and other consumables	138
	£ 335
Overheads	80
Salaries	480
TOTAL	895