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Mode of action of α-methylnoradrenaline on temperature and oxygen consumption in young chickens

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Summary

1. Temperature, oxygen consumption, electromyographic activity, plasma non-esterified fatty acids and blood sugar were estimated in conscious unrestrained young chickens under conditions of thermoneutrality (31° C) and below thermoneutrality (16° C). In some chickens carotid arterial pressure was also recorded.

2. At thermoneutrality, α-methylnoradrenaline lowered temperature and oxygen consumption in intact or chronically vagotomized chicks. α-Methylnoradrenaline was ineffective on temperature in chicks with transection of the brain-stem posterior to the hypothalamus but anterior to the respiratory centre. Hypothermia due to α-methylnoradrenaline was associated with a significant reduction of plasma non-esterified fatty acids but blood sugar was not significantly altered. Lowering of temperature by α-methylnoradrenaline occurred despite vasoconstriction which would hinder heat loss.

3. Temperature and oxygen consumption were reduced by α-methylnoradrenaline in chronically thyroidectomized chicks to the same extent as in intact chicks but recovery did not occur unless the chicks were taken from the metabolism chamber and warmed artificially. In contrast, chronically thyroidectomized chicks given replacement thyroxine were relatively resistant to α-methylnoradrenaline.

4. Oxygen consumption of tissue slices from different parts of the chick's brain, including the diencephalon, was not altered by α-methylnoradrenaline over an extensive dose range. The effects of α-methylnoradrenaline on temperature and oxygen consumption in intact chickens were unlikely, therefore, to be due to depressed metabolism of neurones.

5. In an environment below thermoneutrality (16° C) temperature was considerably reduced and carotid arterial pressure fell 40–50 mmHg. In contrast, electromyographic activity, oxygen consumption and plasma non-esterified fatty acids were markedly raised whereas blood sugar was insignificantly elevated.

6. In experiments at 16° C, α-methylnoradrenaline markedly reduced oxygen consumption although values were still higher than those at thermoneutrality. Temperature fell further, but whereas the reductions in oxygen consumption and temperature were long-lasting, electromyographic activity (shivering) was only transiently diminished. Plasma non-esterified fatty acids were reduced after

α-methylnoradrenaline but not significantly so; blood sugar was not significantly altered. The time-course for recovery of oxygen consumption following α-methylnoradrenaline paralleled recovery from its blood pressure effects but the effect on oxygen consumption was not a consequence of the blood pressure changes. The effects of α-methylnoradrenaline on temperature, oxygen consumption and electromyographic activity were similar to those of another central depressant, pentobarbitone.

Introduction

Catecholamines given intravenously to young chickens lower body temperature and oxygen consumption (Allen & Marley, 1967). A central action has been proposed to account for these hypothermic effects (Allen & Marley, 1967), a proposition strengthened by the finding that catecholamines micro-infused into the hypothalamus of young chickens had similar effects to those obtained when the amines were given intravenously (Marley & Stephenson, 1968, 1969). Catecholamines injected into the third ventricle of adult fowls also have marked hypothermic effects (Grunden & Marley, 1970). Feldberg & Myers (1964, 1965), using cats, had earlier shown that adrenaline or noradrenaline injected into the cerebral ventricles or given by micro-injection into the anterior hypothalamus lowered body temperature.

The present experiments were undertaken to examine further the effects of a particular catecholamine, α-methylnoradrenaline, on body temperature and oxygen consumption in young chickens. α-Methylnoradrenaline was used since substances which have a methyl group attached to the carbon atom immediately adjacent to the basic group are not, or only slowly, deaminated by monoamine oxidase (Blaschko, 1952). Consequently it has a more prolonged action than noradrenaline when given intravenously (Allen & Marley, 1967) or by microinfusion into the hypothalamus (Marley & Stephenson, 1968, 1969) of young chickens or by injection into the third cerebral ventricle of adult chickens (Grunden & Marley, 1970). A preliminary account of the work was given to the British Pharmacological Society (Allen, Garg & Marley, 1969).

Methods

Animals. Rhode Island Red pullets, 1–23 days old, weighing 30–150 g, and adult fowls up to 2·4 kg were used. For the first week the young chickens were kept in a cage thermostatically maintained at 33° to 34° C; subsequently chickens and fowls were kept at 29° to 31° C.

Anaesthesia. For implanting cannulae, electrodes and thermistors, chickens were anaesthetized with halothane (Fluothane, I.C.I.) in oxygen 1·5% v/v, delivered through a Goldman or a VAPOR halothane vaporizer (Marley & Payne, 1964). Halothane was delivered routinely via a mouth tube, but in the case of brain-stem transections the trachea was intubated and the chicken ventilated at 24/min with a respiration pump until the anaesthetic was stopped. For perfusion of a hind limb, the fowl was anaesthetized with phenobarbitone sodium (200 mg/kg) and artificially ventilated.

Operative procedures. These were similar to those described by Allen & Marley (1967). In some chickens a polyethylene cannula was tied into a carotid artery to

record blood pressure or to obtain arterial blood samples. For brain-stem transection, the bone over the cerebellum was removed, the dura incised and the brain-stem transected with a blunt leucotome.

For thyroidectomy, the two thyroid glands, each situated in the root of the neck close to the angle formed by the union of the subclavian and common carotid arteries, together with adherent thymic tissue were dissected away. That the dissected portions were thyroid tissue was later confirmed microscopically. In addition, following testing with α-methylnoradrenaline, some chicks were killed with an overdose of anaesthetic and the operation site examined to confirm removal of thyroid tissue. In sham operated chicks, the same operative procedures were followed as for thyroidectomy but the thyroid glands left intact.

Perfused hind limb. Heparinized blood was drawn from one ischiadic artery through silicone rubber tubing by a roller pump (Saxby, Siddiqi & Walker, 1960) driven by a Servomex Motor Controller and collected in a reservoir of approximately 4 ml capacity. It was then delivered at 15–20 ml/min by the roller pump into the proximal end of the ischiadic artery of the contralateral limb. The reservoir prevented transmission of changes in systemic blood pressure to the perfused limb. Perfusion and systemic carotid pressures were measured by transducers writing out on a Devices pen recorder. Flow rate was adjusted so that perfusion pressure approximated systemic blood pressure.

Post-operative care

After recovery from anaesthesia (5–20 min), chicks were returned to a draught-free recovery box maintained at the same temperature as the home cage. However, chicks with a brain-stem transection were transferred to an oxygen consumption chamber in which they were to be tested the following day. Until the test, the chamber was partially submerged in a water bath at 34° C so that the water surface reached approximately to an inch below its upper margin ; the chamber was not sealed so there was free air exchange. By these means, body temperature was maintained satisfactorily and the period for body temperature to adjust after sealing the chamber at testing was markedly shortened.

Recording procedure

In the following tests, the variables—for example oxygen consumption, temperature, electromyographic activity and blood pressure—were recorded as was injection of drugs or saline and removal of blood samples without handling the chicken or disturbing its external milieu (Allen & Marley, 1967 ; Dewhurst & Marley, 1965).

Tests were not made until at least 24 h after recovery from anaesthesia except in the case of the perfused hind limb, when the fowl remained under anaesthesia for the entire experiment and was then destroyed. In thyroidectomized chicks, tests were made 7 to 13 days after thyroidectomy.

Oxygen consumption. Oxygen consumption was measured as described by Allen & Marley (1967).

Body temperature. Body temperature was recorded by implanted thermistors. The thermistors, type GB32J1 (Radon Industrial Electronics Co.), were prepared and calibrated as described by Allen (1969). They were implanted in the posterior

mediastinum to record core temperature, and the leads brought under the skin and fixed to the cranium with Simplex autopolymerizing resin (Dental Fillings Ltd.). The free ends of the thermistor leads were connected to a linear output thermistor bridge circuit (Allen & Lanworn, 1968) the output of which was fed into a Devices multi-channel pen recorder. A continuous record of temperature was obtained, although the records were plotted graphically at 5 min intervals.

Electromyographic activity. Electromyographic activity was recorded from electrodes implanted in the dorsal muscles of the neck (Key & Marley, 1962). Recordings were made on a Devices pen recorder. The electromyogram was integrated by a modification of the method of Dewhurst & Marley (1965), giving a numerical record plotted graphically at 5 min intervals.

Blood pressure recording. Blood pressure was recorded from a carotid artery, by a Devices blood pressure transducer, on a Devices pen recorder. Blood pressure was recorded continuously in mmHg (1 mmHg≡1·333 mbar) and the results graphed at 5 min intervals.

Blood sugar estimation. Blood sugar content of whole blood was analysed, using the glucose oxidase method (Hill & Kessler, 1961), on an auto analyser (Technicon). Samples, 0·2 ml, were withdrawn from a carotid artery via a polyethylene cannula and stored in a plastic bottle containing sodium iodoacetate. Blood sugar was determined in chicks maintained at environmental temperatures of 31° or 16° C. Four groups, each of six to eight animals, were used. At each environmental temperature, one group served as a control and one group was injected with α-methylnoradrenaline. After acclimatization for 90 min, blood samples were withdrawn at 30 min intervals, five or six samples being taken from each chick. α-Methylnoradrenaline was injected immediately after removal of the 90 min specimen, as was an equal volume of saline in the control group.

Plasma non-esterified fatty acids (NEFA). Chickens were placed in an oxygen consumption chamber at 31° or 16° C. Forty-four chickens in four groups of eleven were used. At each environmental temperature one group received the drug and a second group served as a control. Control and drug treated chicks were kept for 2 h at the respective environmental temperatures. Temperature was recorded and then saline injected via an implanted jugular venous cannula in the control group and α-methylnoradrenaline given intravenously to the other group, the volume of injectate and wash-in being equal for all chicks. After a further hour temperature was again recorded and 1 to 1·5 ml of blood removed from a carotid artery via an implanted polyethylene cannula containing heparin-saline. The blood samples were spun at 3,000 rev/min for 5 min in heparinized centrifuge tubes and the plasma pipetted off and stored in a refrigerator. NEFA were estimated by the method of Duncombe (1963, 1964) except, for better separation of the copper nitrate and chloroform phases, the samples after shaking were stood in a refrigerator for 2 h and then centrifuged at 10,000 rev/min for 15 min. Calibration curves were made using stearic acid (Koch-Light) as described by Duncombe (1963).

Oxygen consumption of brain tissue slices. The effect of α-methylnoradrenaline was tested on metabolism of isolated brain tissue slices from 5–15 days old chickens.

The chickens were decapitated and the dorsal neck muscles and vertebrae trimmed back to the base of the skull. The skull was cut along the sagittal suture

from the posterior fontanelle as far anterior as possible ; cuts were then made from the mid-line along the coronal suture. The four flaps of skull were retracted and the brain removed whole and put into Krebs-Ringer-phosphate buffer solution previously bubbled with oxygen ; the procedure took about 1 min. The solution contained 100 parts NaCl 0·154 M, 4 parts KCl 0·154 M, 3 parts $CaCl_2$ 0·11 M, 1 part KH_2PO_4 0·154 M, 1 part $MgSO_4 \cdot 7H_2O$ 0·145 M and 20 parts 0·1 M phosphate buffer (17·8 g $Na_2HPO_4 \cdot 2H_2O$ + 20 ml 1 N HCl in 1 litre).

Portions of the brain stem, hypothalamus and cerebrum (each weighing 100–240 mg) were then dissected and the remaining brain discarded. The portions of brain were cut into slices 0·0508 mm thick using a tissue chopper described by McIlwain & Buddle (1953). The slices were transferred to a manometric flask containing 2 ml of either Krebs-Ringer solution or Krebs-Ringer solution plus drug. The flasks were run in pairs so that for each sample in drug solution there was a corresponding sample in plain Krebs-Ringer solution. To absorb carbon dioxide, 0·2 ml of 20% KOH solution was added to the centre cup of the flask. The flasks were attached to their respective manometers and placed in a water bath at 38° C with the taps open. Oxygen was passed through the system for 10 min, after which a further 10 min was allowed for equilibrium ; the taps of the flasks were then closed and recordings made for 100 min.

Histology. Brains and thyroid glands were preserved in formol saline. Thyroid tissue was stained with haematoxylin and eosin ; the brain was stained with luxol fast blue and cresyl violet after rapid embedding in celloidin (Inman, 1968).

Range of thermoneutrality. There is a wide range for thermoneutrality in young or adult chickens. The lower critical point for thermoneutrality is the temperature at which shivering develops or oxygen consumption is significantly elevated. In our system shivering, recorded electromyographically and tested on five chicks each at 7, 14 and 21 days, commenced when the air temperature in the chamber was 26·0° ± 0·5° C. In earlier work using the same test situation (Allen & Marley, 1967), however, oxygen consumption was not significantly increased at 25° or 28° C compared with that at 31° and 34° C, but was at 22° C. The development of panting is taken as the upper critical point for thermoneutrality. This occurs in 1 to 22 day chicks at an air temperature of 42·8° C (Randall, 1949). Panting was difficult to observe in our test situation but appeared to occur at about 41° C. The upper and lower critical points for thermoneutrality were therefore taken as 41° and 22° C. Tests at thermoneutrality were therefore made at an environmental temperature of 34° C for chickens with brain-stem transection and, unless otherwise stated, at 31° C for intact chickens. Tests below thermoneutrality were made at an environmental temperature of 16° C.

Drugs. These (with the molecular weights of salts in parentheses) were the hydrochlorides of (−)-α-methylnoradrenaline (220); tyramine (174); mebanazine oxalate [(±)-α-methyl benzyl hydrazine] (220) ; L-thyroxine sodium salt (798) and pentobarbitone sodium. All drugs were weighed up freshly for each injection and dissolved in 0·9% saline immediately before the injection. Doses are given in μmol/kg and injections made intravenously unless otherwise stated.

Results

Tests at thermoneutrality

Tests were made to determine whether the fall in temperature induced by α-methylnoradrenaline was mediated via neural pathways or by humoral factors.

Neural mechanisms

Chickens with a brain-stem transection. To ascertain whether central neural pathways were involved, chickens were tested with a transection posterior to the hypothalamus so placed (Fig. 1) that respiration continued unassisted. Since such chickens maintained body temperature with difficulty, there would be a small gradient between body and environmental temperatures. Consequently, tests were first made in twelve intact chicks aged 8 to 16 days to determine the minimal temperature gradient at which α-methylnoradrenaline had hypothermic effects. Environmental temperature was set between 32° to 36° C, 5° to 7° C lower than that of the chicks. Of the five chicks in which there was a gradient of 7° C, 10 and 20 μmol/kg α-methylnoradrenaline lowered temperature 1° to 2·5° C; of the two chicks in which there was a gradient of 6·5° C, 20 μmol/kg α-methylnoradrenaline reduced temperature by 1° and 2·5° C and 40 μmol/kg produced larger temperature falls; in the remaining five chicks where there was a gradient of 5° to 6° C, α-methylnoradrenaline (20 or 40 μmol/kg) was ineffective. The minimal necessary gradient was therefore taken as 6·5° C.

Of the fifteen chickens tested aged 10 to 16 days and with brain-stem transection, there were only five in which the section was subsequently found to be complete and in which it was possible to maintain a gradient of 6·5° C or larger. α-Methylnoradrenaline (10, 20 and 40 μmol/kg) was ineffective in four of these chicks, but in the fifth a 20 μmol/kg dose lowered temperature from 40·5° to 39·0° C.

Vagotomized chickens. Since the vagi constitute an alternative efferent neural pathway to the spinal cord, tests were made in two chronically vagotomized chicks. As shown in Fig. 2 a fall in temperature and oxygen consumption occurred with 10 to 20 μmol/kg α-methylnoradrenaline, responses within the normal range. The effects in normal chicks were unlikely therefore to be mediated via vagal pathways.

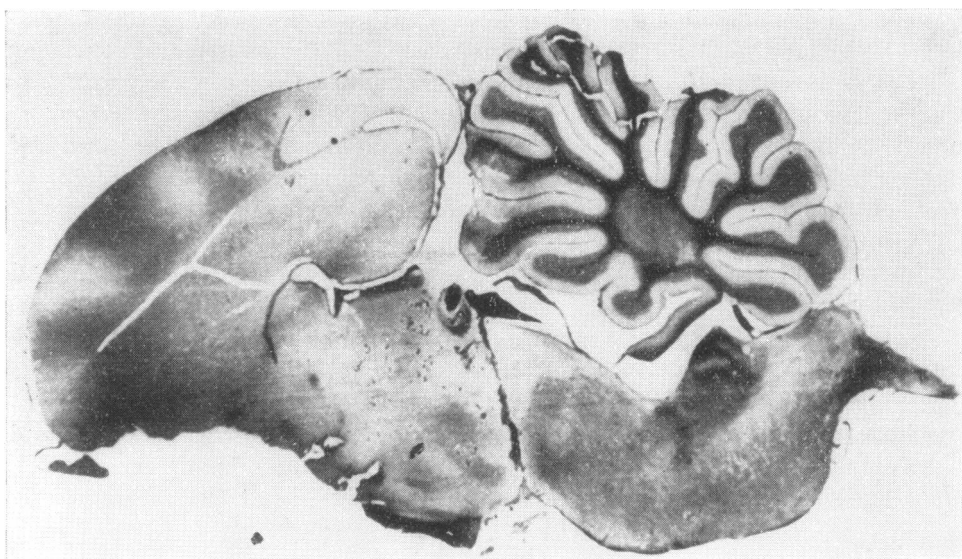

FIG. 1. Paramedian sagittal section of brain stained with luxol fast blue and cresyl violet from an 8-day chick showing transection of the brain-stem immediately posterior to the hypothalamus and optic chiasma but considerably anterior to the respiratory centre.

Oxygen consumption of isolated brain slices. These experiments were made on the assumption that decreased oxygen consumption and temperature following α-methylnoradrenaline might be due to a non-specific depression of neural metabolism. However, for the thirty chick brains tested, α-methylnoradrenaline (0·01, 0·05, 0·1, 0·5 and 1·0 μmol, each dose added to 100–240 mg brain slices in 2 ml Krebs-Ringer solution) did not alter oxygen consumption of slices from diencephalon, brain-stem or cerebrum. Because α-methylnoradrenaline was without effect the results are not tabulated, but a typical result was (for the 0·1 μmol dose) a control mean oxygen consumption of $15{\cdot}98 \pm 1{\cdot}1$ μl oxygen/g per min and $15{\cdot}92 \pm 1{\cdot}0$ μl oxygen/g per min after the drug ($n=6$). As the system was under high oxygen tension, the lack of effect of α-methylnoradrenaline could have been due to its rapid oxidation. This was unlikely because pressor potency, measured in a pithed rat, of a solution of α-methylnoradrenaline stood in the light and bubbled with a 100% oxygen was unimpaired after 90 min and only reduced 10% at 120 min.

Humoral mechanisms

Vasoconstriction with α-methylnoradrenaline. Long-lasting intense pressor effects, presumably due to vasoconstriction, are obtained with α-methylnoradrenaline

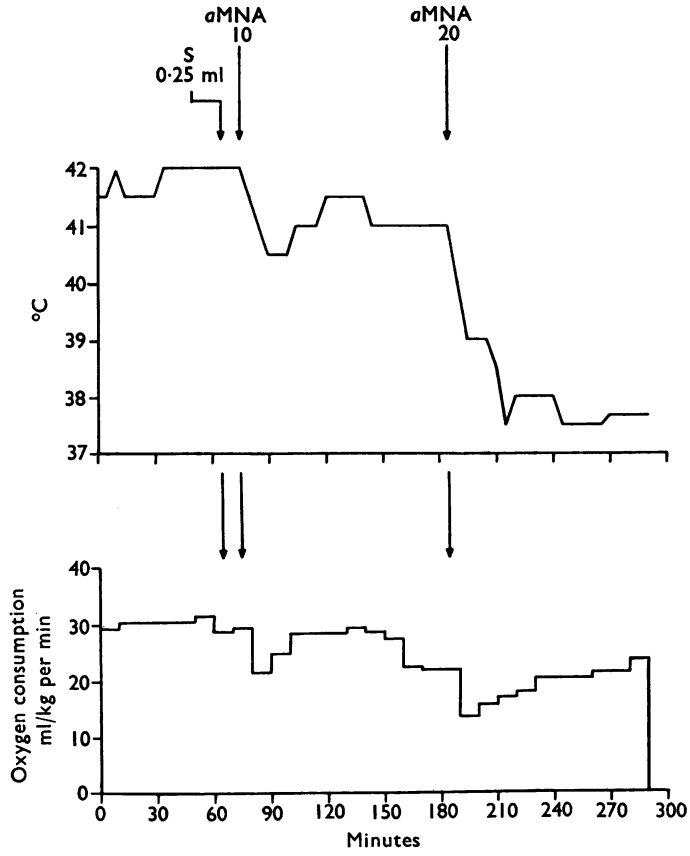

FIG. 2. Graph of temperature and histogram of oxygen consumption in a 13 day vagotomized chick. Oxygen consumption chamber maintained at 31° C. Falls of temperature with 10 and 20 μmol/kg α-methylnoradrenaline (αMNA) within normal range; reduction of oxygen consumption also occurs with each dose.

TABLE 1. *Blood sugar (B.S., mg%) and body temperature (B.T., °C) in control groups and groups treated with α-methylnoradrenaline (20 μmol/kg)*

First sample taken 90 min after acclimatization, and samples subsequently taken at 30 min intervals	Environmental temperature 31° C				Environmental temperature 16° C			
	Control		α-methylnoradrenaline		Control		α-methylnoradrenaline	
	B.S.	B.T.	B.S.	B.T.	B.S.	B.T.	B.S.	B.T.
90	139·3±17·2	39·7±0·7	176·2±13·8	40·2±0·5	164·8±10·9	39·5±0·9	143·6±8·6	38·2±1·2
120	144·6±10·8	39·8±0·4	161·4±20·2	39·0±0·8	172·2±10·6	39·0±1·1	145·6±12·9	36·4±1·2
150	163·0±15·0	39·5±1·0	195·4±19·1	38·6±1·1	174·8±5·4	38·1±1·4	151·3±14·0	35·6±1·2
180	168·0±19·0	39·2±1·1	195·0±22·1	38·0±1·0	177·1±11·1	37·2±1·4	142·6±13·6	34·8±0·9
210	154·0±31·2	39·0±0·7	209·6±17·1	37·1±1·4	177·4±9·9	36·2±1·2	138·0±12·2	33·7±1·0

Each group contained six to eight chickens aged 13–16 days. Values shown are mean±S.E.M. for five or six samples in each chicken. α-methylnoradrenaline injected immediately after removal of 90 min specimen.

in fowls (see Fig. 8A). As vasoconstriction would reduce heat loss, systemic blood pressure and perfusion pressure in a hind limb of anaesthetized fowl were recorded. Intravenous injections of α-methylnoradrenaline (0·25 or 2·5 μmol/kg) raised both perfusion and blood pressure to the same extent and the effects were of the same duration ; thus hypothermia occurred despite coincident vasoconstriction.

Blood sugar and non-esterified fatty acids. The hypothermic effects of α-methylnoradrenaline are probably determined by a change in thermogenesis involving utilization of carbohydrates or fatty acids. Since chickens have a small blood volume, tests were made to ensure that serial removal of blood did not significantly alter blood sugar concentration or body temperature. The results from twenty-six chickens maintained at an environmental temperature of 31° C are summarized in the left side of Table 1. In control tests, blood sugar concentrations were higher in the later samples removed (for example at 180 and 210 min) than those taken immediately after the 90 min acclimatization ; the changes in blood sugar and body temperature were not significant. Blood sugar was raised by α-methylnoradrenaline (20 μmol/kg), but not significantly.

Larger blood volumes were required for measuring non-esterified fatty acids, so only one blood sample was taken from each chicken. The effects of α-methylnoradrenaline were tested in eleven chicks and the values compared with those in eleven control chicks not injected with α-methylnoradrenaline (Table 2). At 31° C, the mean NEFA concentration was 0·88 mequiv./l. in control chicks, but in those given α-methylnoradrenaline (20 μmol/kg) there was a significant decrease ($P<0·05$) 60 min after injection to 0·64 mequiv./litre. Immediately before removal of blood the mean temperature was 36·4° C as against a control of 38·6° C.

Thyroid and recovery from hypothermia due to α-methylnoradrenaline. Six chicks were tested 7 to 13 days after thyroidectomy. Resting temperature and oxygen consumption tended to be lower in these than in sham-operated chicks. In each, the fall of temperature evoked by α-methylnoradrenaline (10 μmol/kg) was of usual intensity but temperature did not recover (Fig. 3A) unless the chick was taken from the chamber and warmed ; the reduction in oxygen consumption was also prolonged. A further 3 thyroidectomized chicks were treated daily with L-thyroxine (3 μg/100 g subcutaneously), a dose found by Singh, Reineke & Ringer (1968) to counteract in chicks the effects of a goitrogen, 1-methyl-2-mercaptoimidazole ; an inadequate or excessive dose led to loss of weight. To ensure adequacy

TABLE 2. *Non-esterified fatty acids (NEFA) (mequiv./l.) and body temperature (°C) in control groups and groups treated with 20 μmol/kg α-methylnoradrenaline at environmental temperatures of 31° C and 16° C.*

Environmental temperature (°C)	Control		α-methylnoradrenaline		
	Body temperature	NEFA	Body temperature	Fall in body temperature after drug	NEFA
31	38·1±0·08	0·88±0·08	38·6±0·21	2·16±0·25	0·64±0·06
16	27·1±0·65	1·55±0·12	31·8±0·4	4·3 ±0·35	1·44±0·19

Values shown are mean±S.E.M. For each of the four groups, eleven chickens aged 13 to 16 days were tested. One blood sample taken from each chicken after 3 h at appropriate environmental temperature. Control saline and α-methylnoradrenaline injected 1 h before removal of blood sample. Temperature measured immediately before injection and at end of 3 h experimental period. The mean change in temperature after saline was less than 0·25° C and is not included.

of dose, sham-operated chicks served as controls. These and the thyroidectomized chicks were weighed daily and since the weight gain in the two groups corresponded, the dose of thyroxine was considered satisfactory. These treated chicks proved moderately resistant to the hypothermic effects of α-methylnoradrenaline. As shown in Fig. 3B, α-methylnoradrenaline (10 or 20 μmol/kg) had insignificant effects on

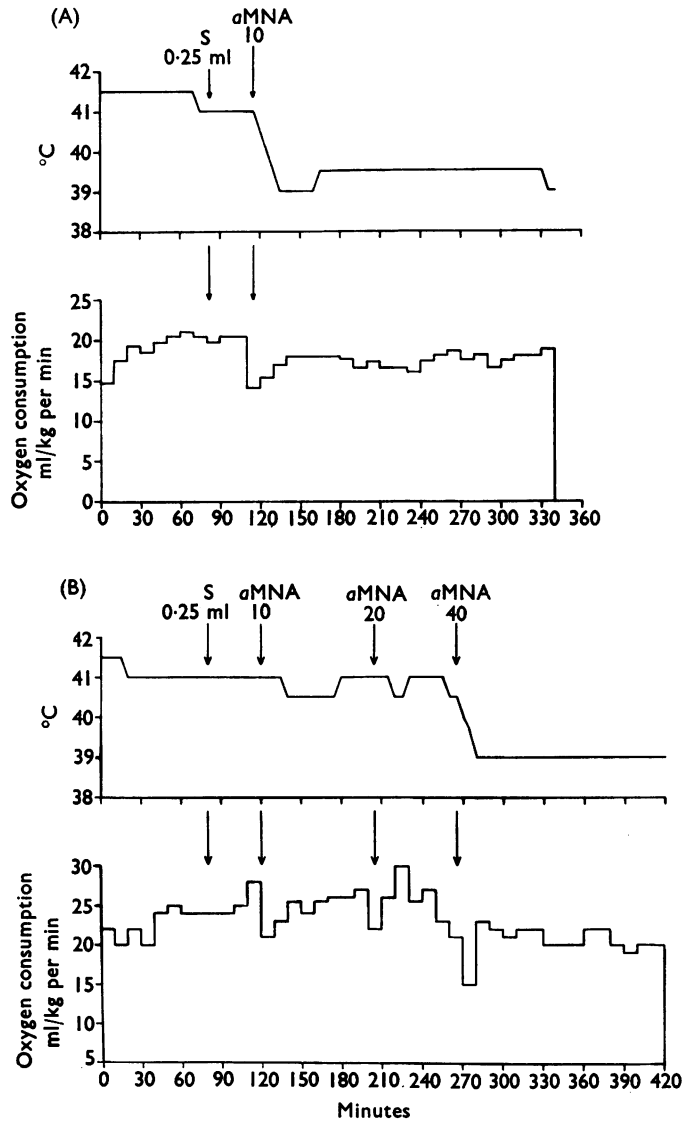

FIG. 3. Graphs of temperature and histograms of oxygen consumption in two chickens aged 15 days both maintained at an environmental temperature of 31° C. (A) Chick thyroidectomized 7 days previously. Note fall of temperature and oxygen consumption after α-methylnoradrenaline (αMNA) 20 μmol/kg without recovery at end of experiment. Temperature eventually recovered after warming chick under a lamp. (B) Chick thyroidectomized 7 days previously and given L-thyroxine 30 μg/kg subcutaneously. Note ineffectiveness of α-methylnoradrenaline 10 and 20 μmol/kg, but lowering of temperature and oxygen consumption by α-methylnoradrenaline 40 μmol/kg. Temperature had not recovered by end of experiment but eventually did so without applying warmth.

temperature and small effects on oxygen consumption. A dose of 40 μmol/kg lowered temperature and oxygen consumption to the same extent as did 10 or 20 μmol/kg α-methylnoradrenaline in intact chicks. Temperature had not recovered by the end of the experiment but did so subsequently without warming the chicken. Thyroxine appeared therefore to play some part in recovery from hypothermia due to α-methylnoradrenaline.

Tests below thermoneutrality

These tests differ from those at 31° C in that shivering may be pronounced. The inter-relation of shivering, measured by integrating electromyographic activity, to body temperature and oxygen consumption at 16° C was first investigated in chickens of various ages to provide a baseline for subsequent drug studies and also because it has been suggested that shivering is not pronounced in young chicks.

Oxygen consumption, temperature and electromyographic activity. Chickens were maintained first in an oxygen consumption chamber at an environmental temperature of 31° C for 60 min and then transferred to one at 16° C for 180 min. An increase of 100% or more in oxygen consumption was considered an adequate response to chilling (16° C) and an increase less than that as an inadequate response. About 30% of chickens aged 1–7 days and a similar proportion aged 8–14 days responded inadequately to chilling (Table 3). In these, the fall in temperature (15·6°–18·9° C) was 2–3 times greater than that (7·4°–7·5° C) for chicks responding adequately. Besides a greater fall in temperature, mean oxygen consumption was reduced in the 1–7 day group responding inadequately and rose only 20·6% in 8–14 day chicks in contrast to increases of 444·3 and 250% respectively in chicks responding adequately in the two groups. There was also a substantially lower mean percentage increase in electromyographic integrals in chicks responding inadequately. The three chicks of the group aged 1–7 days which responded inadequately were aged 6 and 7 days respectively ; the eight chicks which responded adequately included two aged 2 days. Typical results for an adequate response to

TABLE 3. *Mean values for core temperature, oxygen consumption and integrated electromyographic activity in three groups of young chickens in a controlled environment at* 31° *C (left) and mean values for fall in temperature and % increases of oxygen consumption and integrated electromyographic activity in the same three groups of chickens after transfer to an environment at* 16° *C (right)*

		Control (31° C)			N	Chilling (16° C)		
Age (days)	N	Temp °C	Mean values O_2 consumption ml/kg per min	Electromyogram integrals		Max. temp. fall °C	Mean values % increase in O_2 consumption ml/kg per min	% increase in electromyogram integrals
1– 7	11	39·9	19·7	204·9	8	7·5	444·3	1,280·3
					3	18·9	Decreased 8·0	124·4
8–14	19	40·6	24·3	210	14	7·4	250	280
					5	15·6	20·6	59
15–21	9	42·1	26·1	298·5	9	6·5	215·7	161

For the groups of 1–7 and 8–14 day chickens the upper column of figures are for chicks responding adequately at 16° C and the lower column for chicks responding inadequately to chilling. Definitions of adequate and inadequate responses given in text.

chilling are shown in Fig. 4. Oxygen consumption doubled, temperature fell 11° C and integrals of electromyographic activity increased gradually by 600% during chilling. The increased integrals corresponded with increased electromyographic activity consisting of large muscle potentials associated with shivering superimposed on a base-line of raised muscle electrical activity. These potentials appeared after placing the chamber together with the chick in the bath at 16° C but preceded decline of core temperature.

In the drug tests, chicks older than 8 days were used since younger chicks tend to become poikilothermic after α-methylnoradrenaline (Allen & Marley, 1967);

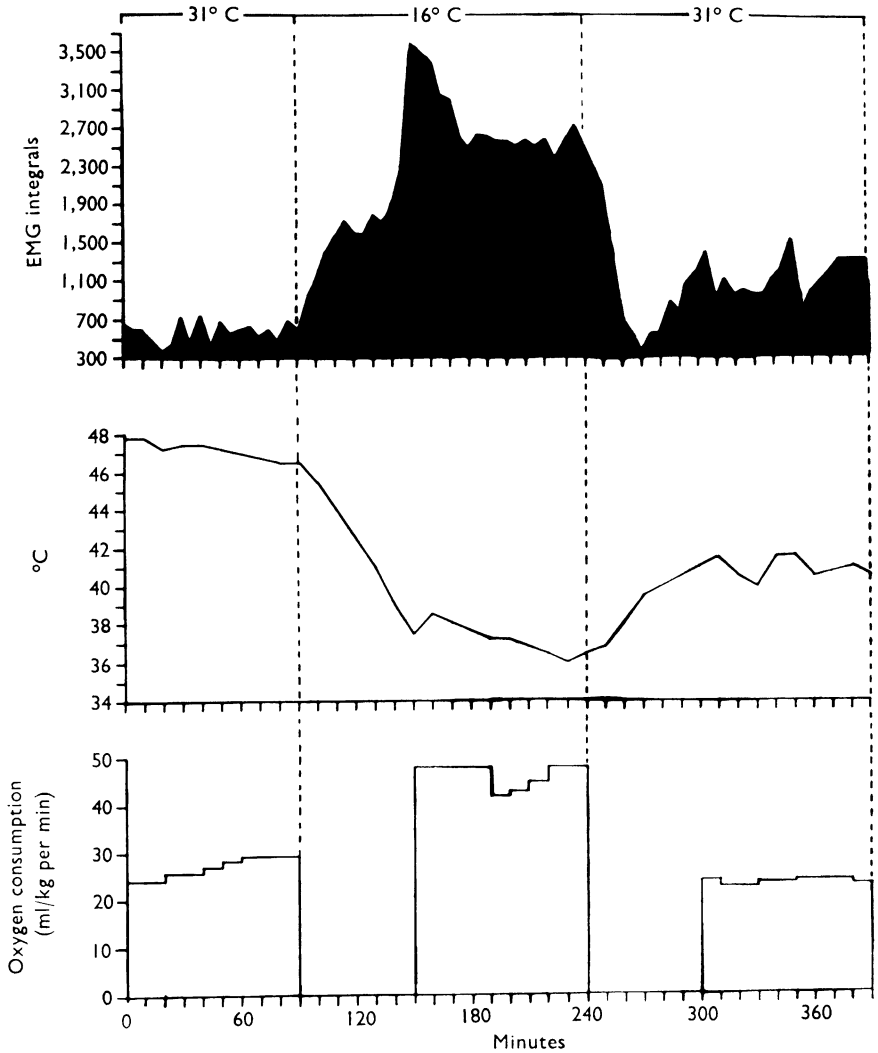

FIG. 4. From above downwards, integrated electromyographic activity, graph of temperature and histograms of oxygen consumption in a 16 day old chick at serial environmental temperatures of 31° C, 16° C and 31° C. Massive increase of electromyographic activity, fall in temperature and increase of oxygen consumption during period below thermoneutrality (16° C). In this and subsequent figures, the breaks between histograms of oxygen consumption are the periods allowed for thermal equilibrium to re-establish after transfer of the oxygen consumption chamber from an environment at 31° C to one at 16° C.

chicks aged 8–15 days which failed to respond adequately to chilling were also excluded.

The effect of α-methylnoradrenaline was examined in six chickens and compared with that of another central depressant, pentobarbitone, in four chicks, each drug injected during the period of chilling. The effects of α-methylnoradrenaline (20 μmol/kg—a dose that elicited sleep) are shown in Fig. 5, which illustrates that besides lowering core temperature a further 5° C and decreasing oxygen consump-

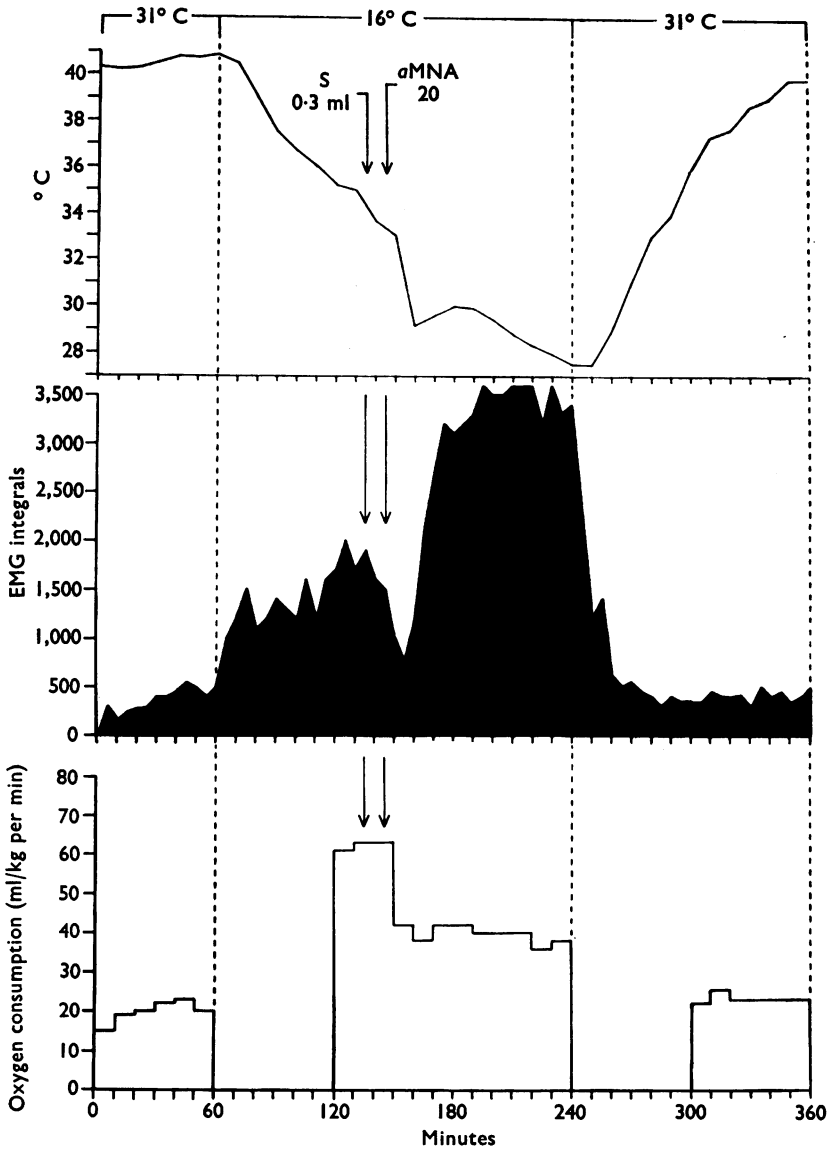

FIG. 5. From above downwards, graph of temperature, integrated electromyographic activity and histograms of oxygen consumption in an 8 day chick at serial environmental temperatures of 31° C, 16° C and 31° C. Note further decrease in temperature, and reduction in oxygen consumption by α-methylnoradrenaline (αMNA) 20 μmol/kg which persists throughout the period at 16° C. In contrast, there is a much briefer reduction in electromyographic activity.

tion from about 60 to 40 ml/kg per min for the remainder of chilling—that is from about three times to twice the control values, the effect on electromyographic activity was evanescent. Thus temperature and oxygen consumption stayed lower for the remainder (90 min) of the period at 16° C, but electromyographic activity had returned to its pre-injection value after 15 min. The interpretation of Fig. 5 is difficult and will be considered in the **Discussion.** Of four chicks given pentobarbitone during chilling (total of 20 mg/kg in two doses over 15 to 20 min—a dose which elicited sleep but not anaesthesia), the electromyogram was briefly reduced in three and abolished in one. The results supported the suggestion that lowering of temperature by α-methylnoradrenaline or pentobarbitone could not be accounted for in nine of the ten chickens by interference with shivering thermogenesis.

Portions of the records of oxygen consumption and of electromyographic activity from which Fig. 5 was derived are shown in Fig. 6. In the control trace (Fig. 6A) from a chicken at an environmental temperature of 31° C, about 1 bubble/min oxygen was utilized as indicated by the interval between the upstroke of the pen and its decline to baseline ; the amplitude of electromyographic activity was 50 μV. When environmental temperature was lowered to 16° C, oxygen consumption increased to about three bubbles/min and electromyographic amplitude rose to 100–150 μV with superimposed 350 μV potentials (left hand section, Fig. 6B). Following α-methylnoradrenaline (20 μmol/kg), oxygen consumption fell to just less than two bubbles/min with reduction of electromyographic amplitude to 50–70 μV and eclipse of superimposed potentials. The delay of 90 s before these changes developed was partly due to slow injection of the drug (over 60 s). Electromyographic potentials increased again after 3 min.

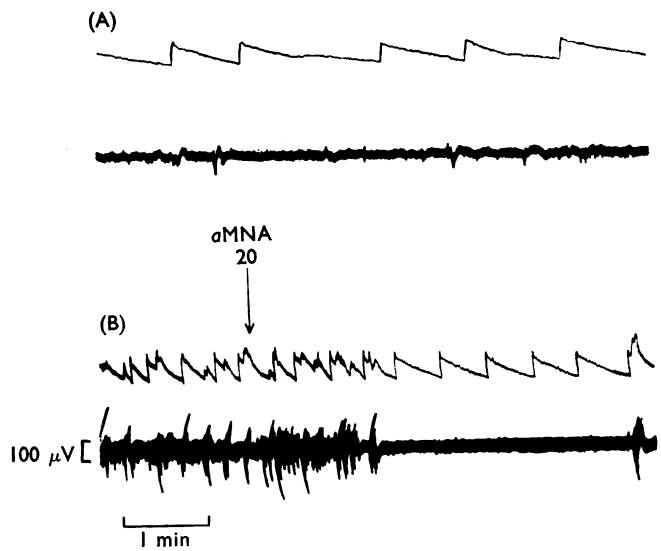

FIG. 6. Trace of oxygen uptake (each bubble of oxygen entering the metabolism chamber indicated by upstroke of pen) and of electromyographic activity from an 8 day chick in an environment at 31° C (A) and at 16° C (B). In B, increased oxygen uptake and augmented muscle potentials with superimposed " shivering " potentials. Note reduction of oxygen uptake and reduction of muscle tone with temporary abolition of " shivering " potentials by α-methylnoradrenaline (αMNA) 20 μmol/kg.

Blood pressure in relation to oxygen consumption and temperature. Tests were made to see whether the fall in oxygen consumption with α-methylnoradrenaline was a consequence of baroreceptor activation secondary to the pressor effects of the drug.

The effect on blood pressure of changing environmental temperature from 31° C to 16° C was first ascertained. On the left and right hand sides of Fig. 7 are the control and recovery values respectively for temperature and arterial blood pressure of a 16 day chick in an environment at 31° C. On lowering environmental temperature to 16° C, as shown in the centre of the figure, oxygen consumption doubled, core temperature fell 8° C and arterial blood pressure declined 40 to 50 mmHg.

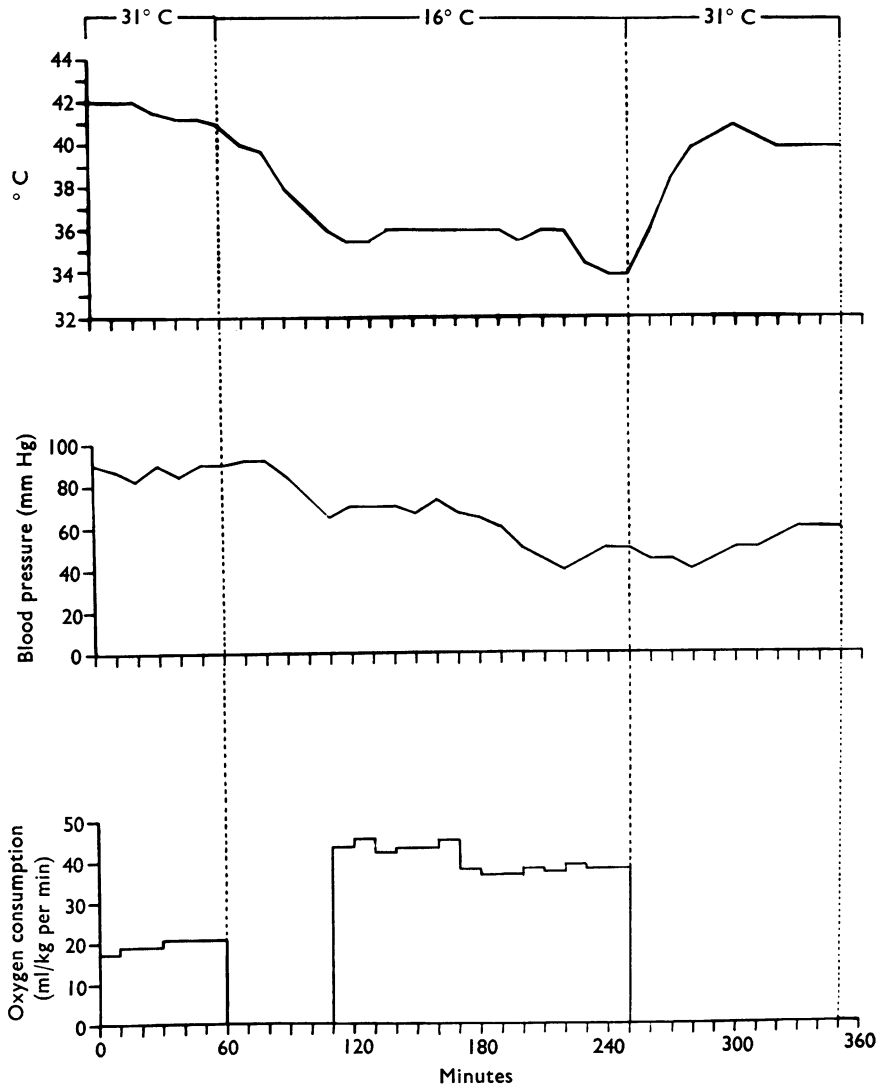

FIG. 7. From above downwards, graphs of temperature, carotid arterial blood pressure and histograms of oxygen consumption in a 16 day chick at serial temperatures of 31° C, 16° C and 31° C. Note fall of temperature and blood pressure with augmented oxygen consumption during the period at 16° C.

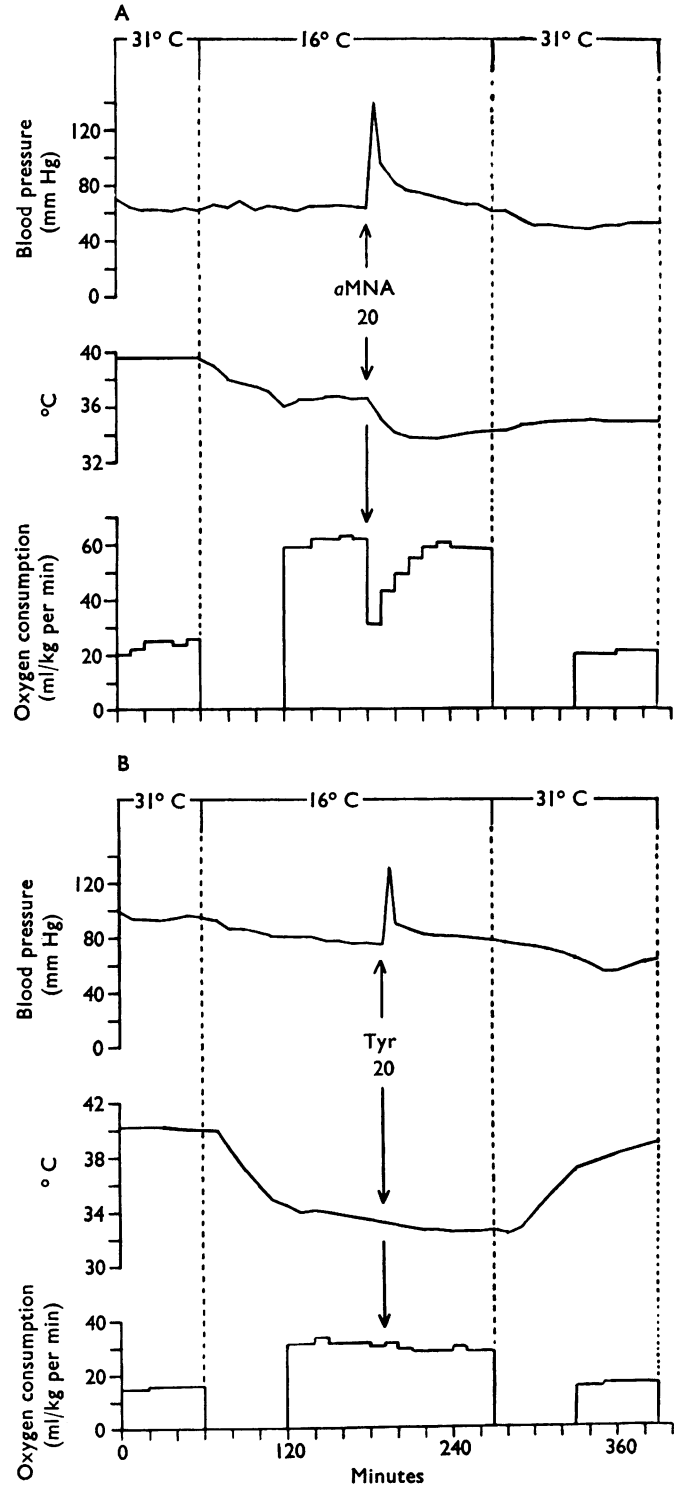

FIG. 8. From above downwards, graphs of blood pressure, temperature and histograms of oxygen consumption for two 15 day chicks at serial temperatures of 31° C, 16° C and 31° C. (A) Rise in blood pressure, decline in oxygen consumption and further fall of temperature induced by α-methylnoradrenaline (αMNA) 20 μmol/kg. Note recovery of oxygen consumption parallels restoration of blood pressure. (B) Chick pretreated with mebanazine (100 μmol/kg, 18 h previously and 1 h before the experiment). Rise in blood pressure induced by tyramine (Tyr) 20 μmol/kg of slightly smaller magnitude but similar duration to that provoked by α-methylnoradrenaline. No effect on temperature or oxygen consumption.

Exposure to cold lowers arterial blood pressure in adult fowls (Rodbard & Tolpin, 1947), but this aspect seems not to have been previously established for recently hatched chicks.

The effect of α-methylnoradrenaline was now tested in five chickens, a typical result being shown in Fig. 8A. Blood pressure was raised on average 70 mmHg by α-methylnoradrenaline (20 μmol/kg), with a return to pre-injection values over the next 74 min. Mean oxygen consumption was reduced 46·8% and mean core temperature 2·5° C. Whereas reduction in temperature persisted for substantially longer than the pressor effect, the peak reduction in oxygen consumption coincided with the peak increase in blood pressure and returned to pre-injection values *pari passu* with the return in blood pressure. Since the decrease in oxygen consumption could be reflexly determined by the rise in blood pressure, the effects were compared with those of another pressor amine, tyramine, which has fewer central effects. Six chickens were pretreated with mebanazine (100 μmol/kg 18 h previously and 1 h before the experiment) to ensure a prolonged pressor response with tyramine (20 μmol/kg). As shown in Fig. 8B, despite a blood pressure rise of similar magnitude and duration to that with α-methylnoradrenaline, oxygen consumption was unaffected and the fall in temperature was not enhanced. The effects of α-methylnoradrenaline on oxygen consumption were unlikely, therefore, to be due to the rise in blood pressure.

Blood sugar and non-esterified fatty acids. In control tests chilling at 16° C and serial sampling of blood did not significantly affect blood sugar concentration (Table 1); nor did serial sampling of blood sugar affect body temperature. Blood sugar concentration was lowered by α-methylnoradrenaline in tests at 16° C compared to an increase at 31° C, although the differences were not significant.

The effects of chilling (16° C) and of α-methylnoradrenaline on NEFA in plasma were studied in twenty-two chicks aged 12–16 days. The results are summarized in Table 2. The mean NEFA level in control animals was 1·55 mequiv./l, significantly greater ($P<0·001$) than control values in animals maintained at 31° C. After α-methylnoradrenaline (20 μmol/kg), the NEFA levels were not significantly reduced despite a significantly greater fall in temperature compared to chicks at 31° C.

Discussion

The fall in temperature and decline in oxygen consumption induced by α-methylnoradrenaline appear to be initiated in the hypothalamus and mediated by pathways descending into the spinal cord. The evidence for this is based on a number of findings. For example, similar phenomena were obtained with micro-infusions of α-methylnoradrenaline into the hypothalamus but not into other areas of the brain (Marley & Stephenson, 1968) and were also elicited by α-methylnoradrenaline given intravenously into chronically decerebrated chicks in which the hypothalamus was subsequently shown to be intact (Marley & Stephenson, unpublished). In the present experiments, hypothermia was not evoked by α-methylnoradrenaline in four of the five chickens tested with a complete transection of the brain-stem posterior to the hypothalamus. A fall in temperature and oxygen consumption was obtained in chickens with an intact neuraxis but with the vagi chronically divided. The responses to α-methylnoradrenaline were more likely to be due to modulation of hypothalamic activity than to depressed hypothalamic metabolism

because oxygen consumption of diencephalic brain-slices was unaltered by α-methylnoradrenaline. The latter was added in doses ranging from one equivalent to the amount calculated to be in the brain, assuming α-methylnoradrenaline to be distributed equally throughout the body, to the entire dose given into an intact chick.

Whether the effects of α-methylnoradrenaline on body temperature and oxygen consumption were mediated through sympathetic or parasympathetic nerves from the spinal cord is not known and the fact that they were abolished by phenoxybenzamine whether α-methylnoradrenaline was given intravenously (Allen & Marley, 1967) or into the hypothalamus (Marley & Stephenson, 1968) far from implicates peripheral sympathetic pathways. The conclusions apply to chickens tested in a thermoneutral environment (31° C) but are likely to apply for situations below thermoneutrality (16° C). Nor is there any reason for supposing that altered secretion of pituitary hormones played any part in the temperature fall, although, in rabbits, release of thyrotrophic hormone is inhibited following adrenaline injection into the mamillary bodies (Harrison, 1961). Even if inhibition had been instantaneous, there would still be sufficient tissue or circulating thyroxine for heat production.

Since the half-lives of thyroxine and tri-iodothyronine are $22{\cdot}5 \pm 1$ h in chickens (Tata & Shellabarger, 1959), these substances were unlikely to be present in chicks thyroidectomized 7–13 days previously. Although the fall of temperature due to α-methylnoradrenaline was no greater in thyroidectomized than in intact chicks, temperature did not recover, suggesting that thyroxine may be required for recovery. This lack of recovery was reminiscent of the tendency to poikilothermia observed following α-methylnoradrenaline in chicks younger than 8 days and attributed to immature thermoregulatory mechanisms (Allen & Marley, 1967). A further pointer to thyroxine involvement was the increased resistance to hypothermic doses of α-methylnoradrenaline in chronically thyroidectomized chickens maintained on thyroxine.

Increase in muscle tone precedes shivering in response to lowered environmental temperature (Burton & Edholm, 1955). In birds, the shivering mechanism develops simultaneously with homeothermy (Odum, 1942), and compatible with this, shivering and increased electromyographic activity were more conspicuous in older chicks. Increase in muscle tone and shivering preceded the fall in body temperature; this implies the presence of thermogenic receptors in the skin and mediation of the thermogenic response via the central nervous system (Freeman, 1967). A fall of body temperature occurred after α-methylnoradrenaline or pentobarbitone in addition to that due to chilling despite augmented electromyographic activity. It occurred also in the presence of vasoconstriction elicited by α-methylnoradrenaline in the hind limb. The hind limbs are an important source of heat loss in the fowl, metabolic rate being 40% higher in erect than in squatting fowls (Deighton & Hutchinson, 1940). Vasoconstriction would reduce heat loss from skin and muscles of the hind limbs, the muscles contributing both to core and shell temperatures (King & Farner, 1961).

α-Methylnoradrenaline produced sleep at the same time it lowered body temperature, but the temperature fall exceeded that of 0·5° to 1·0° C usually found during sleep under thermoneutral conditions. One must suppose that heat production was interfered with by a central action of α-methylnoradrenaline. A hint as to the means whereby this could be induced was obtained from experiments made at 16° C.

As shown in Fig. 5, oxygen consumption increased threefold during chilling, an increase halved by α-methylnoradrenaline. Presumably, the residual increase of oxygen consumption was associated with shivering which continued and increased despite temporary abatement after α-methylnoradrenaline. The viscera and skeletal muscles contributed equally to the chick's weight and both are sources of metabolic heat. Since shivering and presumably muscle heat production were unaffected by α-methylnoradrenaline, the halving of the increase in oxygen consumption would be due to reduced visceral metabolism. The concentration of blood sugar and of NEFA were therefore estimated under the different experimental conditions.

The effects of changes in body temperature on blood sugar apparently differ between young and adult chickens. In adults, blood sugar and body temperature are directly related, blood sugar declining as body temperature decreases and rising as body temperature increases (Rodbard, 1947). In chicks, blood sugar was unaffected by changes in body temperature (Freeman, 1967). In our experiments, blood glucose was not significantly altered by falls in body temperature evoked either by α-methylnoradrenaline or by changes in environmental temperature; indeed, blood sugar increased as body temperature fell.

Fowls utilize lipids rather than carbohydrate for heat production (Freeman, 1967). At thermoneutrality, lowering of temperature by α-methylnoradrenaline was accompanied by a significant reduction of NEFA, findings compatible with the idea that fat metabolism and hence heat production were reduced as a consequence of a central action of α-methylnoradrenaline. Exposure to a cold environment increased NEFA as well as lowering temperature (Freeman, 1967). This was confirmed in our experiments, but although NEFA were again lowered by the same dose of α-methylnoradrenaline, the reduction was not significant. Presumably, chilling introduces other neurogenic and metabolic factors not present at thermoneutrality.

Non-shivering thermogenesis in young chickens has been attributed to activation of β-adrenoceptors by a sympathetic amine (Wekstein & Zolman, 1968). Homeothermy shown by young chicks to chilling was also attributed by these authors to high blood concentrations of catecholamines induced by "cold stress"; they concluded that in such circumstances exogenous catecholamines would have little effect. The present experiments and earlier work (Allen & Marley, 1967; Marley & Stephenson, 1968, 1969) do not support these conclusions, since catecholamines acting at peripheral α- or β-adrenoceptors and given intravenously or into the hypothalamus profoundly lowered temperature and oxygen consumption, particularly at environmental temperatures below thermoneutrality; moreover, their effects were abolished by an antagonist at α-adrenoceptors (phenoxybenzamine) but not by one (propranolol) at β-adrenoceptors.

In newborn and cold-adapted mammals, noradrenaline is thermogenic and there is strong evidence that it is the mediator responsible for non-shivering thermogenesis in response to cold (Moore & Underwood, 1963; Scopes & Tizard, 1963). In newborn mammals heat production has been ascribed to an action of noradrenaline on brown fat (Dawkins & Hull, 1964). In young chickens the mediator is unknown, but on the basis of our results it is unlikely to be noradrenaline. Freeman (1967) came to similar conclusions because of a lack of effect of hexamethonium on the metabolic response of 1 day chicks to cold; moreover young chicks do not contain brown fat (Freeman, 1967) and noradrenaline does not cause lipolysis (Carlson, Liljedahl, Verdy & Wírsen, 1964).

In conclusion, at thermoneutrality lowering of temperature and oxygen consumption by α-methylnoradrenaline are consequences of its action on the hypothalamus and apparently require an intact neuraxis for their mediation. The effects might be determined by reduction in fat metabolism and occur despite vasoconstriction which hinders heat loss ; thyroxine is in some way implicated in recovery. Below thermoneutrality, the effects of α-methylnoradrenaline can be assumed to be similarly mediated, even when accompanied by shivering which increases heat production. Because the relation to fat metabolism was not clear-cut there must be other unrecognized determinants. Work is in progress to define these.

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