*Br. J. Pharmac. Chemother.* (1968), **31**, 568–577.

# THE EFFECT OF LIPOLYTIC HORMONES AND THEOPHYLLINE ON HEAT PRODUCTION IN BROWN ADIPOSE TISSUE *IN VIVO*

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(*Received August* 15, 1967)

Brown adipose tissue has been shown to be a site of heat production in hibernating mammals during arousal (Smalley & Dryer, 1963; Smith & Hock, 1963; Lyman & Taylor, 1964; Joel, 1965) and in new-born rabbits (Dawkins & Hull, 1963; Hull & Segall, 1965a), new-born guinea-pigs (Brück & Wünnerberg, 1965) and adult rats (Donhoffer, Sárdy & Szegváry, 1964) during cold exposure. There is good evidence that this response is mediated by the release of noradrenaline at sympathetic nerve endings in brown adipose tissue (Hull & Segall, 1965b). *In vitro* noradrenaline accelerates the hydrolysis of triglyceride in brown adipose tissue. Two hormones which recently have been shown to have similar lipolytic action *in vitro*—glucagon and corticotrophin—stimulate heat production in brown adipose tissue of new-born rabbits *in vivo* (Heim & Hull, 1966). The object of the present investigation was to extend these observations by examining the effect of these and other lipolytic hormones and drugs on heat production in brown adipose tissue of new-born guinea-pigs and young rats, as well as new-born rabbits.

## METHODS

New-born rabbits and guinea-pigs less than 24 hr old were anaesthetized with ether, and a fine polyethylene catheter was inserted into a branch of the external jugular vein. After recovery from the anaesthetic the animal was placed in a metabolism chamber. Sprague-Dawley rats age 6 to 8 weeks (75–130 g) were anaesthetized with urethane (1 g/kg intraperitoneally) and ether, and a tail vein was cannulated. The rats, still anaesthetized, were then placed individually in the metabolism chamber for measurements of the rate of their oxygen consumption. Both adrenal glands were removed from some rats immediately before preparing them for the metabolism chamber.

The oxygen consumption was measured by a closed circuit method identical to that used by Heim & Hull (1966), and based on the method of Scopes & Tizard (1963). The chamber and most of the circuit was immersed in a water-bath whose temperature was controlled to $\pm 0.1°$ C. Water-bath temperature was maintained at 35° C for the new-born rabbit studies, at 32° C for the guinea-pig and 31° C for the rat. These environmental temperatures are at the lower end of the thermal neutral range for each species. Oxygen consumption is expressed in terms of dry gas at s.t.p. In each animal fine thermocouples were inserted under the skin over the interscapular brown adipose tissue, under the skin over the lumber muscles, and in the colon, 2–3 cm from the anus. Temperatures from these three sites were recorded continuously by a Cambridge slow recorder.

Drugs and hormones were infused into the external jugular or tail vein at a constant infusion rate of 0.02 ml./min for a period of 10 min. The doses of (−)-noradrenaline (noradrenaline bitartrate,

Winthrop Lab.), serotonin (5-hydroxytryptamine creatine sulphate, May and Baker Ltd.), hydrocortisone (hydrocortisone sodium succinate, Glaxo Laboratories Ltd.) and theophylline ethylene diamine (aminophylline, Boots Pure Drug Co. Ltd.) are expressed in terms of base. Doses of corticotrophin (Crookes Laboratories Ltd.), thyrotrophin (Thytropar, Armour Pharmaceutical Co.), vasopressin (Pitressin, Parke, Davis & Co.) are expressed as international units and glucagon hydrochloride (Eli Lilly & Co.) in terms of the salt. Human growth hormone prepared from fresh-frozen pituitaries by the Raben procedure had a potency of 1–2 i.u./mg. In every experiment noradrenaline (intravenously 2 $\mu$g/kg min for 10 min) was infused. If the animal failed to show a significant increase in the rate of its oxygen consumption and in the temperature over its brown adipose tissue the preparation was discarded. No more than five infusions were given to each animal, and no more than two agents were tested in any individual experiment. The mean rate of oxygen consumption over a 6 min period, when the response was at a maximum, was used as a measure of the response.

## RESULTS

All three mammals responded to noradrenaline infusion (intravenously 2 $\mu$g/kg min for 10 min) by a large rise in the rate of their oxygen consumption which was accom-

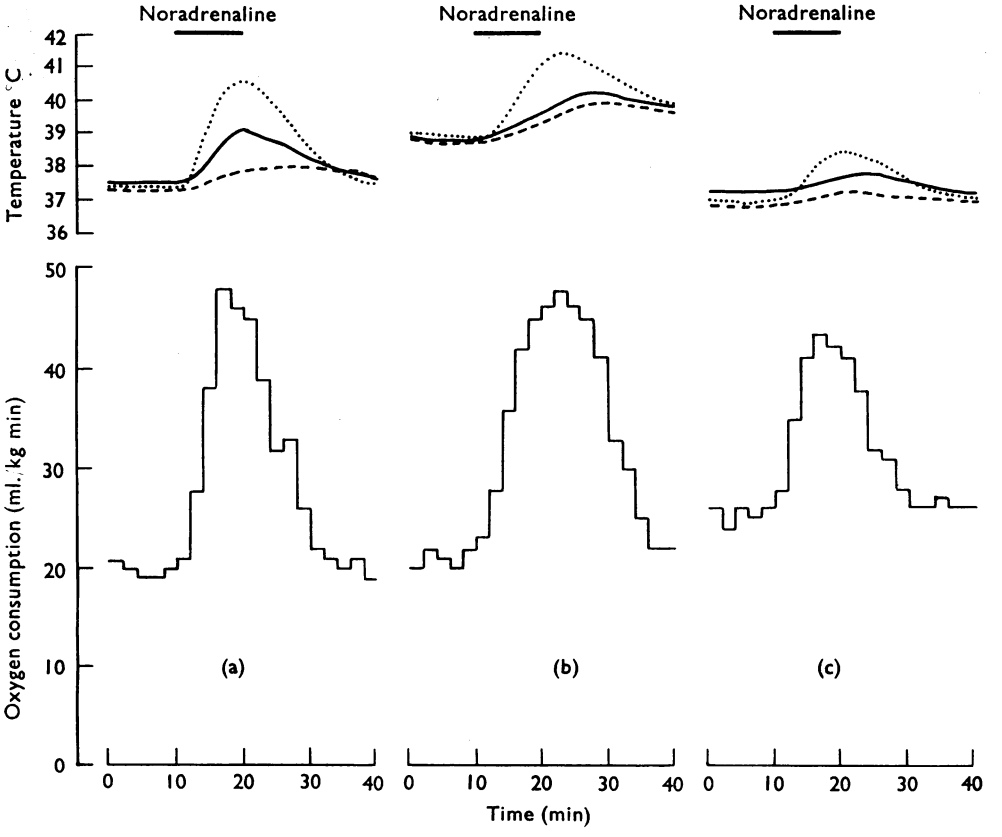


Fig. 1. The effect of intravenous infusions of noradrenaline (2 mg/kg min for 10 min) on the rates of oxygen consumption and subcutaneous temperatures over brown adipose tissue (dotted line) in the colon (continuous line) and over muscle (interrupted line) in (a) rabbit (age 0 days, weight 83 g), (b) guinea-pig (age 0 days, weight 118 g) and (c) rat (age 6 weeks, weight 100 g). Environmental temperatures were: rabbit, 35° C, guinea-pig 32° C, and rat 31° C.

panied by an immediate large increase in the temperature over their brown adipose tissue (Fig. 1, Table 1). In young rats bilateral adrenalectomy did not abolish this response (Table 1).

TABLE 1

THE EFFECT OF NORADRENALINE INFUSION (2 μG/KG MIN FOR 10 MIN) ON THE RATE OF OXYGEN CONSUMPTION OF UNANAESTHETIZED NEW-BORN RABBITS AND GUINEA-PIGS AND OF ANAESTHETIZED YOUNG INTACT AND ADRENALECTOMIZED RATS AND ON THE SUBCUTANEOUS TEMPERATURE OVER THEIR BROWN ADIPOSE TISSUE

The measurements of oxygen consumption in this and subsequent tables are expressed as ml./kg min of dry gas at S.T.P. All values are expressed as mean ±S.E. with range bracketed.

| Species | No. | Weight (g) | Oxygen consumption (ml./kg min) Initial | Oxygen consumption (ml./kg min) During noradrenaline | Increase in temp. over brown adipose tissue (°C) |
|---|---|---|---|---|---|
| Rabbit | 100 | 71·2±1·0 (53–100) | 19·8±0·4 (10·8–35·4) | 37·7±0·86 (16·8–62·0) | 1·86±0·07 (0·3–3·3) |
| Guinea-pig | 19 | 93·7±5·8 (63–144) | 26·2±1·2 (17·6–33·8) | 47·3±2·8 (26·0–70·4) | 1·45±0·14 (0·5–2·5) |
| Intact rat | 15 | 95·3±3·8 (75–130) | 26·9±0·64 (22·9–20·4) | 42·7±1·5 (33·8–53·0) | 0·97±0·13 (0·2–2·0) |
| Rat after bilateral adrenalectomy | 6 | 105·8±3·3 (100–120) | 25·5±0·7 (22·5–27·6) | 35·8±1·9 (30·3–42·4) | 0·65±0·1 (0·2–1·0) |

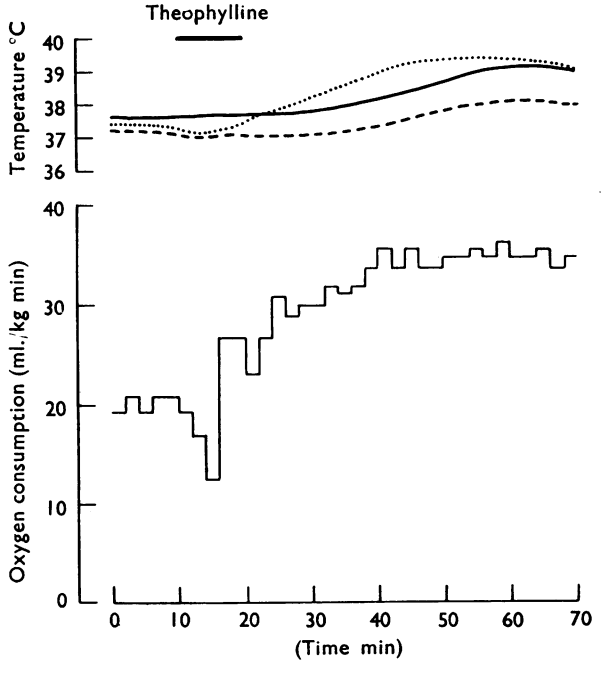


Fig. 2. The effect of intravenous infusion of theophylline (4 mg/kg min for 10 min) on the rate of oxygen consumption and subcutaneous temperatures over brown adipose tissue (dotted line), in the colon (continuous line), and over muscle (interrupted line), in a rabbit (age 0 days, weight 83 g). Environmental temperature was 35° C.

*New-born rabbits*

Infusions of glucagon (4 μg/kg min for 10 min) and corticotrophin (1 i.u./kg min for 10 min) have previously been shown to be calorigenic in new-born rabbits (Heim & Hull, 1966). The following hormones were infused intravenously for 10 min and did not cause either a rise in rate of oxygen consumption or an increase in the temperature over brown adipose tissue: human growth hormone (2 and 250 μg/kg min, 7 rabbits), thyrotrophin (0.01 i.u./kg min, 10 rabbits; 0.1 i.u./kg min, 8 rabbits; 1.0 i.u./kg min, 13 rabbits), hydrocortisone (0.1, 1.0, 10 and 100 mg/kg min, 9 rabbits), vasopressin (0.002, 0.02 and 0.2 i.u./kg min, 8 rabbits) and 5-hydroxytryptamine (5-HT; 2, 20 and 200 μg/kg min, 6 rabbits). In fact, infusion of vasopressin and 5-HT in the larger doses depressed the rabbits' oxygen consumption and caused a fall in the temperature over brown adipose tissue. Infusion of theophylline (2 to 4 mg/kg min for 10 min in 9 rabbits) caused a prolonged calorigenic response (Fig. 2, Table 2). In 7 new-born rabbits

TABLE 2

THE EFFECT OF THEOPHYLLINE INFUSION (2 TO 4 MG/KG MIN FOR 10 MIN) ON THE RATE OF OXYGEN CONSUMPTION OF UNANAESTHETIZED NEW-BORN RABBITS AND GUINEA-PIGS AND OF ANAESTHETIZED YOUNG INTACT RATS AND ON SUBCUTANEOUS TEMPERATURE OVER THEIR BROWN ADIPOSE TISSUE

| Species | No. | Weight (g) | Oxygen consumption (ml./kg min) | | Increase in temp. over brown adipose tissue (°C) |
|---|---|---|---|---|---|
| | | | Initial | 45 min after theophylline infusion | |
| Rabbit unanaesthetized | 9 | 72·1±4·2 (48–83) | 19·2±1·3 (13·3–24·0) | 29·7±2·5 (22·0–44·0) | 0·84±0·15 (0·3–1·7) |
| Guinea-pig unanaesthetized | 5 | 88·4±7·9 (73–118) | 26·8±2·6 (17·6–34·0) | 47·6±4·3 (34·4–60·5) | 1·18±0·35 (0·5–2·5) |
| Intact rat urethane 1 g/kg I.P. | 7 | 94·4±4·8 (72–107) | 27·1±0·94 (23·8–29·5) | 41·5±2·2 (31·6–50·0) | 0·3±0·09 (0·0–0·7) |

propranalol (5 mg/kg) was given intravenously over a 10-min period 35 min after theophylline; there was an immediate fall in the rabbits' rates of oxygen consumption and in the temperature over their brown adipose tissue (Fig. 3, Table 3).

*New-born guinea-pigs*

Glucagon, like noradrenaline infusion, stimulated heat production in brown adipose tissue of new-born guinea-pigs (Table 4), but corticotrophin given intravenously in a dose of 1 i.u./kg min for 10 min did not. A larger dose of corticotrophin (3.0 i.u./kg min) was invariably fatal in new-born guinea-pigs. As in the new-born rabbit, theophylline infusion caused a prolonged rise in the rate of the new-born guinea-pigs' oxygen consumption and in the temperature over their brown adipose tissue (Table 2).

*Young rats*

Infusions of glucagon (8 μg/kg min for 10 min) and corticotrophin (3.0 i.u./kg min for 10 min) stimulated heat production in brown adipose tissue of young rats (Tables 4 and 5), but infusions of human growth hormone (200 μg/kg min for 10 min, 6 rats) and thyrotrophic hormone (1 i.u./kg min for 10 min, 6 rats) did not. After bilateral

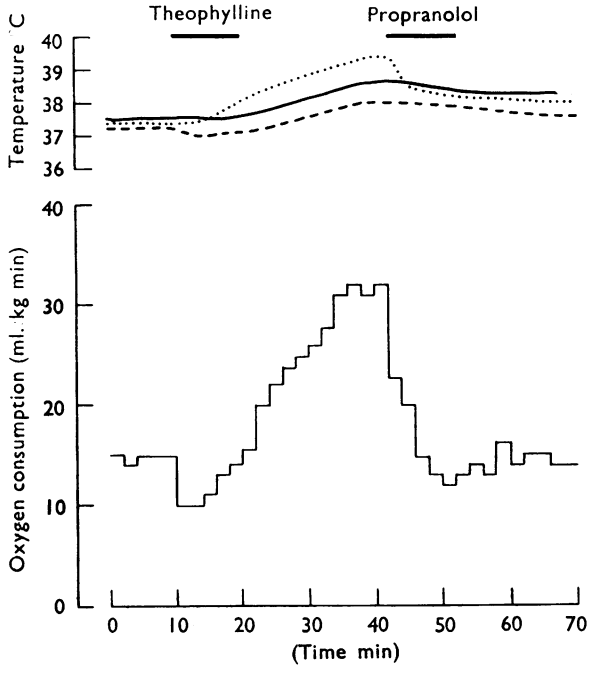


Fig. 3. The effect of intravenous infusion of propranalol (0.5 mg/kg min for 10 min) 25 min after infusion of theophylline (4 mg/kg min for 10 min) on the rate of oxygen consumption and subcutaneous temperatures over brown adipose tissue (dotted line), in the colon (continuous line), and over muscle (interrupted line), in a rabbit (age 0 days, weight 72 g). Environmental temperature was 35° C.

TABLE 3

THE EFFECT OF PROPRANALOL (5 MG/KG) ON THE CALORIGENIC RESPONSE OF UNANAESTHETIZED NEW-BORN RABBITS TO INFUSION OF THEOPHYLLINE (2–4 MG/KG MIN FOR 10 MIN)

| | No. | Weight (g) | Oxygen consumption (ml./kg min) Initial | 35 min after theophylline | 45 min after theophylline | Change in temp. °C over brown adipose tissue from 35 min to 45 min |
|---|---|---|---|---|---|---|
| (a) Control | 9 | 72·1±4·2 (48–87) | 19·1±1·3 (13·3–24·0) | 28·7±2·3 (20·9–42·0) | 29·7±2·5 (22·0–44·0) | (+) 0·07±0·03 ((−) 0·1–(+) 0·2) |
| (b) Propranalol (0·5 mg/kg min infused for 10 min for 35–45 min after theophylline infusion) | 7 | 70·4±5·4 (55–100) | 15·5±0·96 (11·5–18·2) | 26·6±1·6 (20·5–32·4) | 15·9±0·69 (13·8–18·3) | (−) 0·76±0·1 ((−) 1·2–(−) 0·4) |

adrenalectomy the young rats still responded to infusions of noradrenaline (Table 1), glucagon (Table 4), and corticotrophin (Table 5), by an increase in rate of oxygen consumption and a rise in temperature over brown adipose tissue. Similarly, the calorigenic response to cold exposure in young rats was not abolished by removal of both adrenal glands. The rate of oxygen consumption increased in six adrenalectomized rats from 25.5±0.7 (mean±S.E.M.) ml./kg min at 31° C to 35.7±1.5 ml./kg min 60 min after the temperature was reduced to, and maintained at 20° C. The temperatures over

TABLE 4

THE EFFECT OF GLUCAGON INFUSION (RABBITS 4 μG/KG MIN, GUINEA-PIGS AND RATS 8 μG/KG MIN) ON THE RATE OF OXYGEN CONSUMPTION OF UNANAESTHETIZED NEW-BORN RABBITS AND GUINEA-PIGS AND OF ANAESTHETIZED YOUNG INTACT AND ADRENALECTOMIZED RATS AND ON THE SUBCUTANEOUS TEMPERATURE OVER THEIR BROWN ADIPOSE TISSUE

| Species | No. | Weight (g) | Oxygen consumption (ml./kg min) Initial | Oxygen consumption (ml./kg min) After glucagon infusion | Increase in temp. over brown adipose tissue (°C) |
|---|---|---|---|---|---|
| Rabbit* unanaesthetized | 12 | 86±6·4 | 20·2±1·2 (15·0–27·0) | 38·5±1·8 (27·0–51·0) | 1·7±0·4 (0·5–3·8) |
| Guinea-pig unanaesthetized | 9 | 88·1±7·3 (63–130) | 27·8±1·8 (21·0–37·4) | 41·6±3·3 (27·9–54·6) | 0·89±0·25 (0·0–2·1) |
| Intact rat (urethane 1 g/kg I.P.) | 7 | 102·1±5·2 (92–130) | 27·8±0·9 (24·1–31·0) | 38·4±1·5 (35·4–45·6) | 0·40±0·21 (0·2–0·7) |
| Bilateral adrenalectomy rat (urethane 1 g/kg I.P.) | 6 | 105·8±3·3 (100–120) | 25·5±0·7 (23·2–27·6) | 33·7±2·9 (25·3–42·8) | 0·48±0·13 (0·2–1·0) |

* Values reported by Heim & Hull (1966).

TABLE 5

THE EFFECT OF CORTICOTROPHIN INFUSION (RABBITS AND GUINEA-PIGS 1 I.U./KG MIN, RATS 3 I.U./KG MIN FOR 10 MIN) ON THE RATE OF OXYGEN CONSUMPTION AND ON THE SUBCUTANEOUS TEMPERATURE OVER THE BROWN ADIPOSE TISSUE OF UNANAESTHETIZED NEW-BORN RABBITS AND GUINEA-PIGS AND OF ANAESTHETIZED YOUNG INTACT AND ADRENALECTOMIZED YOUNG RATS

| Species | No. | Weight (g) | Oxygen consumption (ml./kg min) Initial | Oxygen consumption (ml./kg min) After corticotrophin infusion | Increase in temp. over brown adipose tissue (°C) |
|---|---|---|---|---|---|
| Rabbit* unanaesthetized | 7 | 65·0±3·2 | 19·8±1·1 (13·0–23·0) | 34·4±1·2 (29·0–41·0) | 1·6±0·2 (0·9–2·8) |
| Guinea-pig unanaesthetized | 10 | 86·6±2·1 (63–130) | 28·7±1·7 (21·0–37·4) | 29·6±2·1 (16·1–36·9) | (−) 0·07±0·12 (−0·6–0·5) |
| Intact rat (urethane 1 g/kg I.P.) | 8 | 101·6±4·9 (86–130) | 26·8±0·84 (23·6–30·4) | 38·4±1·4 (32·2–43·8) | 0·46±0·08 (0·2–0·7) |
| Bilateral adrenalectomy rat (urethane 1 g/kg I.P.) | 6 | 105·8±3·3 (100–120) | 25·5±0·70 (23·2–27·6) | 33·5±2·4 (25·7–40·0) | 0·32±0·13 (0·0–0·9) |

* Values reported by Heim & Hull (1966).

brown adipose tissue rose above the colonic temperatures, although both temperatures fell. The rate of fall over the 60 min period was less over brown adipose tissue ($P<0.05$). There was no significant difference between the responses of the intact and adrenalectomized anaesthetized rats to cooling from an environmental temperature of 31° C to 20° C.

## DISCUSSION

There is now strong evidence that during cold exposure the rate of heat production in brown adipose tissue is controlled by the sympathetic nervous system and that noradrenaline is the mediator at the nerve endings (Dawkins & Hull, 1964 ; Hull & Segall, 1965b). It has been suggested that noradrenaline regulates heat production by controlling the intracellular supply of free fatty acids. The hypothesis rests largely upon the finding that addition of noradrenaline to adipose tissue *in vitro* increases the rate of hydrolysis

of the stored triglyceride to fatty acids by activating tissue lipases. On this hypothesis it might be expected that other hormones and drugs which stimulate hydrolysis of triglyceride in adipose tissue *in vitro* will stimulate heat production in brown adipose tissue *in vivo*. Joel (1966) showed that glucagon and corticotrophin when added to brown adipose tissue from adult rats caused a large increase in the hydrolysis of triglyceride in the tissue and a rise in the rate of the tissues' oxygen consumption. Thyrotropic hormone, although stimulating hydrolysis of triglyceride in white adipose tissue from adult rats, had comparatively little effect on brown adipose tissue even in relatively large doses. Heim & Hull (1966) found that intravenous infusions of glucagon and corticotrophin stimulated heat production in the brown adipose tissue of new-born rabbits and in the present investigation glucagon was shown to be calorigenic in new-born guinea-pigs and glucagon and corticotrophin in young rats. It is difficult to draw conclusions from our finding that corticotrophin did not stimulate heat production in guinea-pigs, for doses equivalent to those used in the rabbit and rat were obviously toxic to the guinea-pig. Thyrotropic hormone was not calorigenic in young rats or new-born rabbits. To this extent the investigations *in vitro* and *in vivo* are in agreement.

It is possible that, *in vivo*, glucagon and corticotrophin produce their calorigenic effect indirectly. For example, it has been shown that they both stimulate the release of catecholamines (Sarcione, Back, Sokel, Mehlman & Knoblock, 1963; Smith, Paoletti & Brodie, 1962). However glucagon and corticotrophin infusions still stimulated heat production in brown adipose tissue after bilateral adrenalectomy in young rats and after propranolol infusion (5 mg/kg) in new-born rabbits (Heim & Hull, 1966). Propranolol, an adrenaline antagonist, blocks the calorigenic response to catecholamine infusions and to cold exposure, so glucagon and corticotrophin do not act by stimulating the release of catecholamines either from the adrenal medulla or from the sympathetic nerve endings in the tissue. The possibility that glucagon produced its effect by stimulating the release of insulin is unlikely in view of the finding that infusions of insulin are not themselves calorigenic in new-born rabbits (Hull, unpublished). Equally, the finding in the present investigation that infusions of hydrocortisone were not calorigenic in new-born rabbits and that infusion of corticotrophin still stimulated heat production in young rats after bilateral adrenalectomy, demonstrates that corticotrophin was not calorigenic by virtue of its action on the adrenal cortex. It seems reasonable to conclude that glucagon and corticotrophin, like noradrenaline, act directly on brown adipose tissue.

Catecholamines, glucagon and corticotrophin also stimulate lipolysis in white adipose tissue *in vitro* and *in vivo*, although there is considerable variation from species to species (Shafrir & Wertheimer, 1965). This property they share with many other hormone preparations. Some of these—growth hormone, vasopressin and 5-hydroxytryptamine—were chosen in the present investigation to see whether or not they stimulated heat production in brown adipose tissue of new-born rabbits. They did not. Unfortunately, firm conclusions cannot be drawn from the experiments in which hormones prepared from the adult of one species had no effect when infused into the young of another. More suitable preparations are not at present available. Infusions of vasopressin and 5-HT in large doses, caused a fall in the rate of the new-born rabbit's oxygen consumption and the temperature over brown adipose tissue. A similar phenomenon was seen during the first few minutes of an adrenaline infusion (Hull, 1964). It is probable that the fall

in oxygen consumption is secondary to the effects of these hormones on bronchial or vascular smooth muscle, resulting in a fall in the oxygen supply to metabolically active tissues including brown adipose tissue. This phenomenon would also mask any calorigenic effect that these hormones might have.

One biochemical link common to noradrenaline, glucagon and corticotrophin is cyclic adenosine 3′, 5′-phosphate (cylic 3, 5-AMP). All three hormones stimulate an increase in the content of 3, 5-AMP in white adipose tissue *in vitro* and this ubiquitous nucleotide has been shown to activate a tissue lipase, as well as phosphorylase, in adipose tissue (Rizack, 1965). Xanthine derivatives, in particular theophylline, have the interesting property that they inhibit the enzyme phosphodiesterase which inactivates 3, 5-AMP (Butcher & Sutherland, 1962), and it has been suggested that many of the pharmacological properties of xanthine derivatives are a consequence of this biochemical reaction (Ritchie, 1965). In adult rats injection of theophylline produces a rise in plasma free fatty acids which reaches a maximum about 45 min after injection (Brodie, Davies, Hynie, Krishna & Weiss, 1966). In our experiments theophylline stimulated a large rise in the rate of oxygen consumption of new-born rabbits, guinea-pigs and young rats; this was accompanied by a large and persistent rise in the colonic temperature and an even greater rise in the temperature over brown adipose tissue. Some, at least, of the extra oxygen consumed by the animals was being used in brown adipose tissue to produce heat. It is tempting to conclude that the lipolytic response in adult rats and the calorigenic response in brown adipose tissue are secondary to an increase in the 3, 5-AMP content of white and brown adipose tissue respectively. However, the calorigenic response to theophylline was blocked after propranolol injection. This rather suggests that theophylline produces its effect *in vivo* by exciting the sympathetic nervous system rather than by inhibiting phosphodiesterase activity in adipose tissue. An alternative interpretation is that the increase in tissue content of 3, 5-AMP, induced by theophylline, could be noradrenaline dependent and that propranolol inhibits theophylline induced calorigenesis by an indirect action on 3, 5-AMP activity.

In conclusion, it has been shown that hormones which stimulate lipolysis and oxygen consumption in brown adipose tissue *in vitro* in general stimulate heat production in brown adipose tissue *in vivo*. On the other hand many other hormones which are lipolytic in the sense that they accelerate the rate of hydrolysis of triglyceride in white adipose tissue *in vitro* and cause a rise in plasma free fatty acids *in vivo* could not be demonstrated to stimulate heat production in brown adipose tissue *in vivo*. It will be of interest to learn if any of these hormones stimulate lipolysis in brown adipose tissue *in vitro*.

SUMMARY

1. The effect of intravenous infusions of various hormone preparations and drugs on the rate of oxygen consumption of unanaesthetized new-born rabbits and guinea-pigs, and anaesthetized young rats, and on the temperature of their brown adipose tissue was measured.

2. In unanaesthetized new-born rabbits intravenous infusions of the following agents did not stimulate heat production in brown adipose tissue: thyrotrophin, human growth hormone, vasopressin, hydrocortisone and serotonin. On the other hand theophylline

infusion caused a large increase in the rate of the rabbits' oxygen consumption and temperature of their adipose tissue. The response to theophylline was blocked by intravenous injection of propranolol.

3. In unanaesthetized new-born guinea-pigs intravenous infusions of noradrenaline, glucagon and theophylline, but not corticotrophin, stimulated heat production in their brown adipose tissue.

4. In anaesthetized young rats intravenous infusions of noradrenaline, corticotrophin and glucagon and cold exposure stimulated heat production in brown adipose tissue. Removal of both adrenal glands did not abolish these responses. Intravenous infusions of theophylline were calorigenic in young rats but infusions of thyrotrophin and human growth hormone were not.

5. It was concluded (a) that the calorigenic effects of glucagon and corticotrophin are not dependent on the presence of the adrenal glands and (b) that agents which are lipolytic *in vitro* may stimulate heat production in brown adipose tissue *in vivo*, but that this is not invariably so.

We wish to thank Dr. G. S. Dawes for his support and advice, Mr. A. Ryder for technical assistance and Dr. W. M. Hunter, M.R.C. Clinical Endocrinology Research Unit, for the supply of human growth hormone.

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