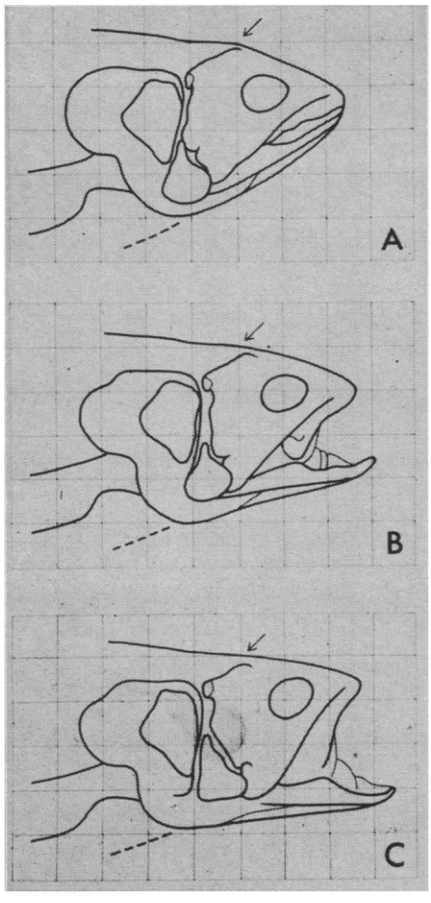

Fig. 1. Head of *Latimeria chalumnae* with mouth closed (*A*), with mouth open in intermediate position (*B*), and with mouth fully open (*C*). Arrows indicate the position of the intracranial joint; the the grid is scaled to 4 cm.

ment of the otico-occipital division of the skull relative to the trunk was observed during operation of the intracranial articulation. In fact the range of potential dorso-ventral movement of the head upon the trunk is narrow, although there is considerable possibility of lateral movement.

It is interesting that the posterior angle of the gape is filled by a complicated whorled fold of skin which presumably prevents food from escaping from the corners of the mouth when the gape is fully opened. One may surmise that a similar fold of tissue must have existed in the fossil Rhipidistia, in which the length of the jaws and extent of the gape were even greater than in coelacanths.

One may distinguish two separate modes of operation of the jaws in *Latimeria*. Firstly, the gape may be opened and closed through an angle of approximately 22 deg and the cheeks expanded and contracted, with a small displacement of the ethmosphenoid (through approximately 8 deg) dorsally from the "resting" position. Arrangement of the ethmosphenoid and palate is such that this amount of displacement of the ethmosphenoid does not produce appreciable forward movement of the quadrate and lower jaws. Further, in this situation simple adduction of the lower jaws by the adductor mandibulae musculature seems to be sufficient to return the ethmosphenoid to the resting position. In this sequence the mechanism of the skull in the feeding and breathing movements is little different from that of other bony fishes.

The second mode of jaw operation is more complicated. The complex arrangement of the intracranial joints, the subcephalic musculature, and the complicated interconnection of the palate, jaws, and hyoid arch (*6*) allow further increase of the gape from the first position. In the process the lower jaws themselves are depressed only through a further 11 deg, but the action of the coraco-mandibular muscles is transferred to the quadrate region, the ethmosphenoid is rotated upward through a further 7 deg, and the whole lower-jaw complex is extended forward.

Operation of the adductor mandibulae muscles alone is apparently not enough to close the mouth when the skull is in this position; closure can be accomplished only by simultaneous action of the mandibular adductors and the subcephalic muscles. Clearly, therefore, the antagonist of the subcephalic muscle system is the coraco-mandibular system that depresses the lower jaws. This second mechanism seems to add little to capability of expansion of the branchial chamber and is probably primarily a feeding mechanism. The action of the subcephalic muscles in retracting the ethmosphenoid must very greatly increase the power of the bite.

The functional significance of intracranial articulation in *Latimeria*, and doubtless in all fossil crossopterygian fishes, is that it can greatly increase the angle of the gape and the general mobility of the jaws; and it greatly increases the power of the mandibular adductor system.

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References and Notes

1. The Coelacanthini and Rhipidistia are classified together as the Crossopterygii.
2. J. Millot and J. Anthony, *Anatomie de* Latimeria chalumnae (Centre National de la Recherche Scientifique, Paris; vol. 1, 1958; vol. 2, 1966).
3. A review by K. S. Thomson of the evidence concerning intracranial articulation in fossil crossopterygian fishes is in press (*Proc. Linnean Soc. London*). Many authors have ascribed a shock-absorbing function to the intracranial articulation, but at least with respect to the Rhipidistia, in which the lower jaw is firmly connected to the otico-occipital through the hyomandibular, this theory is untenable.
4. Caught off Iconi, Grand-Comore, on 14 March 1966, the specimen was conveyed to Peabody Museum, Yale Univ., where it was numbered 1482 in the Fish Collection.
5. The angle of gape was measured from the tip of the snout, to the jaw articulation, to the tip of the mandible.
6. J. Millot and J. Anthony, *Anatomie de* Latimeria chalumnae (Centre National de la Recherche Scientifique, Paris, 1958), vol. 1, fig. 20.
7. I thank Barbara Moss and Algis Taruski for technical assistance, John Howard for photographic assistance, and D. V. Anderson, U.S. Consul-General in Marseilles, for overseeing transportation of the specimen.

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Bipiperidyl Mustard, A New Obesifying Agent in the Mouse

Abstract. *Bipiperidyl mustard, after conversion to its bis-cyclic immonium ion form, has been found to cause rapid and extensive lipid deposition in mice given single doses of the drug. The obesity appears to result from an alkylation reaction; the responses of the treated animals to food restriction and realimentation resemble those observed in gold thioglucose–treated mice; also in analogy to gold thioglucose, the bipiperidyl mustard effects can be counteracted by sulfhydryl compounds. These observations are suggestive of a similarity of mode of action between the alkylating agent and gold thioglucose, a conclusion which is supported by preliminary findings of ventromedial lesions in the hypothalamus of the bipiperidyl mustard–treated mice.*

N,*N*′-bis-(β-chloroethyl)-4,4′-bipiperidine (BPM) was incubated at *p*H 9 (borate, 0.1*N*) at 37°C for 1 hour, and injected intraperitoneally into 20-g female $C_3H \times DBA/2$ mice. Initially the mice went through a period of intoxication and weight loss lasting 3 to 6 days. This was followed by a period of very rapid lipid accumulation lasting for 2 to 3 weeks and producing weight gains up to 20 g (Fig. 1). The next phase was characterized by continued slow weight gain until a plateau was reached 3 to 4 months after injection. The final maximum body weights were 60 to 70 g (three to four times control weight).

Dosage was 5 to 50 mg/kg. The LD_{50} for female C3D2 hybrid mice is approximately 30 to 35 mg/kg. The initial intoxication, subsequent rates of weight

Table 1. Percent lipid and body composition of bipiperidyl mustard–treated obese mice. Animals were given a single intraperitoneal dose (25 mg/kg) and sacrificed at 6 weeks. Carcass lipids were analyzed by exhaustive $CHCl_3$-methanol extraction; organs, by alcohol-ethyl ether extraction of homogenates. Dry weights were obtained on residues from lipid extraction. Expt., experimental; cont., control.

Organ	Wet wt. (g)	Dry wt. (g)	Lipid (g)	Lipid (%)
Carcass				
Expt.	31.2	5.83	13.84	45
Cont.	21.5	5.23	3.53	17
Skin				
Expt.	8.1	1.74	1.91	24
Cont.	5.7	2.26	0.47	8
Liver				
Expt.	2.05		.162	7.9
Cont.	0.90		.028	3.1
Spleen				
Expt.	.13		.0045	3.5
Cont.	.08		.0025	3.2

gain, and final body weights were proportional to the dose (Fig. 1). Treated animals were refractory to additional drug administration prior to the plateau in weight gain, irrespective of the initial dose level. Several strains of mice were tested and all showed a positive response to the drug. The order of response, C3H > C3H×DBA/2 > DBA/2 > Swiss, suggests the possibility of genetic effects in the pure strains and the hybrid.

The obese animals were hyperphagic. They consumed 5.0 to 5.5 g of ration per day, in contrast to 1.5 to 1.7 g per day for littermate controls. Treated animals appeared normal. They were sleek, alert, agile, and active. During and immediately after the initial period of intoxication the treated animals were extremely sensitive to food restriction; pair feeding at the control level of food intake produced continued weight decline and a high incidence of morbidity. In contrast, when obesity was well established (4 to 6 weeks after treatment), the obese animals withstood complete food deprivation for 7 days with only a 10-percent mortality, and upon realimentation, rapidly deposited new tissue and returned to their prestarvation weight levels.

Aside from the obesity, no gross pathology was found. Histological examination revealed no evidence of damage or abnormality in liver, spleen, heart, lung, kidney, or adrenals (*1*). Of the internal organs only liver and spleen were increased in size as compared to controls, and only liver showed significant increase in total lipids (7.9 percent compared to 3.1 percent lipid in controls). The increased liver lipids did not account for the total increase in liver weight: both spleen and liver were hypertrophic with respect to nonfat body weight.

BPM itself has proved inactive as an agent producing lipid deposition (*2*). Apparently it reacts to release Cl^- and become a doubly cyclized form which is pharmacologically active. Evidence that the active agent is the bis-cyclic derivative is provided by studies of the effects of time of cyclization and of *p*H on drug activity. Kinetic studies involving pre-incubation of BPM at 37°C in 0.1*N*, *p*H 9.0 borate buffer for varying time periods up to 60 minutes, followed by intraperitoneal injection of the cyclized drug, resulted in weight gains proportional to the time of pre-incubation. If weight gain is equated to 100-percent reaction, the fractional weight gains can be plotted semilogarithmically against time of incubation at *p*H 9. The observed slope is approximately 0.05 min^{-1}. Rao and Price (*3*), studying the kinetics of cyclization of BPM, have shown the reaction to be complete in 45 to 60 minutes, and a rate constant of about 0.05 min^{-1} can be estimated from their data. We have also shown that the weight gain is a function of the concentration of the conjugate base form of the drug in the range *p*K ± 1 *p*H unit (that is, *p*H 7.0 to 9.0; *p*K = 8.0). Further evidence on the requirement for the bis-cyclic immonium form is provided by the failure of the following compounds with or without pre-incubation to provoke lipid deposition and/or weight gain: *N,N'*-bis-(β-hydroxethyl) bipiperidine, *N,N'*-bis-(dimethyl)-bipiperidine, and *N*-(β-chloroethyl) piperidine (*4*). Since obesifying action has not previously been reported as an attribute of polyfunctional β-chloroethyl amine alkylating agents (*5*) our data imply that this unique physiological action results from the specificity of the bis-cyclic ethylene immonium bipiperidine structure.

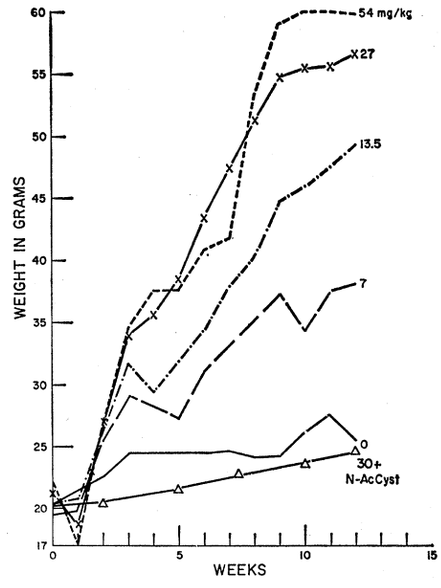

Fig. 1. Weight gain in C3D2 female mice related to dose of BPM and to *N*-acetylcysteine (1500 mg/kg) administered 15 minutes in advance of BPM (single intraperitoneal dose in milligrams per kilogram as indicated).

Analysis of the body composition of the obese mice shows that the entire gain in weight can be accounted for as methanol $CHCl_3$-extractable lipid components. The data of Table 1 clearly illustrate this, as well as the lack of disturbance in the nonfat dry weight produced by drug administration. The highly specific nature of the physiologic effects can be seen, since if the lipid content is excluded, the nonfat dry weight/body weight ratio is approximately the same for treated and control mice. Accompanying the synthesis

Table 2. Incorporation of U-^{14}C-glucose and ^{14}COOH-acetate into tissues of bipiperidyl mustard–treated C3H × DBA/2 mice. Each mouse received a 25 mg/kg intraperitoneal dose of BPM. One and 3 weeks after treatment, experimental mice were given 1 μc of the glucose or 1 μc of the acetate and were sacrificed after 2 hours.

Tissue	U-^{14}C-glucose			^{14}COOH-acetate		
	Control	Experimental		Control	Experimental	
		1 wk	3 wk		1 wk	3 wk
	Percentage ^{14}C incorporation per gram of tissue					
Adipose tissues	0.45	0.96	0.60	2.8	8.5	1.4
Liver	1.73	1.85	1.82	0.33		0.05
Muscle	0.85	0.67	0.74			
Heart	1.70	1.06	.97	.06		.07
	*Total percentage of ^{14}C incorporation into adipose tissues**					
	0.35	0.83	5.7	4.0	8.5	13.3
	Percentage of ^{14}C in CO_2†					
	34	62	53	57	50	38

* Percent ^{14}C/g × g of lipid per mouse. † Respiratory CO_2 collected for 120 minutes.

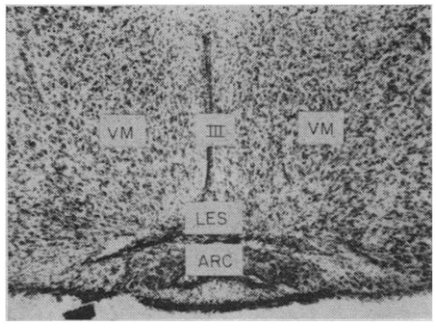

Fig. 2. Section of the brain of C3D2 female mouse, 3 months after intraperitoneal injection of 25 mg/kg of BPM. VM, ventromedial nucleus; III, third ventricle; LES, lesion; ARC, arcuate nucleus. [Courtesy Dr. R. A. Liebelt, Baylor University]

of lipids, there is initially a great acceleration in the oxidation of glucose, amounting to 100 percent at 1 week and 50 percent at 3 weeks after drug administration (Table 2). Despite the increased oxidation of glucose in vivo, there is also an increased fixation of glucose carbon into adipose tissues. Similar increases are also noted for ^{14}C-acetate 1 week after drug treatment, but there is subsequently a decline in the specific but not the total in vivo utilization of acetate. Preliminary investigations, with standard adipose tissue assay systems, indicate that there is no decline in the in vitro utilization of glucose or acetate for fatty acid synthesis during the period of rapid weight gain (0 to 5 weeks postdrug) but that subsequently these in vitro activities do decline significantly.

Since the obesity-inducing action of BPM seems to depend on the availability of the cyclic immonium derivative, it seemed reasonable to expect that the physiological effects result from alkylation reactions (*6*). To test this assumption we pretreated animals with *N*-acetylcysteine, a protective agent which we have demonstrated to effectively antagonize nitrogen mustard (*7*). Preprotection with *N*-acetylcysteine almost completely abolishes the obesifying effect of the BPM; only at high doses (40 to 50 mg/kg) is minor residual activity observed. As can be seen from the data (Fig. 1), weight gains on doses of BPM up to 30 mg/kg are restricted to the control level after *N*-acetylcysteine administration. In such experiments the LD_{50} of BPM is displaced from 30 to 35 mg/kg to ~45 mg/kg, a protection factor of 1.5, whereas the weight gain at 45 mg/kg is not significant. Thus, at equivalent biological doses as regards toxicity, the obesifying activity is virtually eliminated in the *N*-acetylcysteine treated animal, suggesting a specific antagonism between the -SH protective agent and the bipiperidyl mustard. Similar observations have also been made for the only other known chemical obesifying agent, gold thioglucose (*8*).

In summary, in numerous ways, the action of BPM seems to resemble that of gold thioglucose, which is known to produce hypothalamic lesions in the mouse (*9*). This resemblance has now been strengthened by the demonstration of hypothalamic lesions in BPM-treated mice. The lesions (Fig. 2) are located in the ventromedial and arcuate regions and appear very similar to those observed in gold thioglucose obese mice. Should the functional and anatomical resemblances be confirmed by further work now in progress, the chemical relations disclosed for both BPM and gold thioglucose suggest that -SH bearing sites in the hypothalamus may be targets for both drugs and may be involved in the appetite regulatory mechanism (*8*).

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References and Notes

1. The histologic examinations were performed on 60-g female obese C3H×DBA/2 mice by Dr. Philip Custer, pathologist, the Presbyterian Hospital, Philadelphia, Pa., to whom our appreciation is expressed.
2. K. Gerzon, Eli Lilly & Co., private communication. BPM, originally synthesized by Gerzon, was also shown by his group to be inactive against experimental tumors, a finding which we have verified for both the original drug and its cyclized derivative.
3. K. Prasada Rao and C. C. Price, unpublished observations submitted in partial fulfillment of requirements for the Ph.D. degree by one of the authors (K.P.R.).
4. We are deeply indebted to Dr. Charles C. Price for supplies of these drugs, as well as for the bipiperidyl mustard used in these experiments.
5. W. C. J. Ross, *Biological Alkylating Agents* (Butterworth, London, 1962); E. Hirschberg, *Cancer Res.* **23**, Pt. 2, 521 (1963).
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7. R. J. Rutman and F. S. Lewis, unpublished observations.
8. J. Mayer, *New England J. Med.* **274**, 662 (1966).
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10. We are deeply indebted to Dr. R. A. Liebelt (Baylor University) for the preparation and evaluation of the brain sections, a representative specimen of which is included in this report, and in particular, for the identification of the brain lesion.
11. Supported by PHS grant CA05295.

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Increased Cardiovascular Reactivity to Angiotensin Caused by Renin

Abstract. *After a short period of tachyphylaxis, there is a marked and sustained enhancement of pressor responses to renin and angiotensin during chronic administration of renin.*

Subcutaneous injections of rat or hog renin to uninephrectomized rats elicit hypertension and vascular disease (*1*). Measurements of blood pressure obtained by sphygmography of the tail showed that the increase in pressure takes place only after a latent period of 2 to 3 days. This is similar to the result obtained by infusing subpressor doses of angiotensin; a period of normotension also precedes hypertension (*2, 3*). Since it is unlikely that hypertension is due to the accumulation of circulating angiotensin (*4*), the possibility that changes in cardiovascular reactivity could account for the hypertensive response was tested in conscious animals by directly recording the pressor responses to daily injections of renin.

Female Sprague-Dawley rats (150 to 200 g) maintained on commercial chow and tap water were used. After plastic tubing (PE 10) had been inserted into the lower aorta through the femoral artery, the animals were placed, for a 2-day recovery period, in harnesses which permitted free movements within the cage. They were then uninephrectomized to reduce renal antipressor activity. Mean arterial pressure was recorded during 8 hours daily with a Statham P23Db transducer connected to a Sanborn recorder. Crude hog or rat renin (40 Goldblatt units) was injected subcutaneously once every 24 hours. The results obtained by injecting these two preparations were similar and will be presented together. In some animals, test doses of angiotensin II (12.5 ng) and renin (0.05 units) in saline were injected intravenously through an intrajugular catheter. Daily base pressures were those recorded every morning, approximately 20 hours after a previous injection of renin. The experiments lasted up to 9 days. Data were obtained from 12 animals.

The first subcutaneous injection of renin elicited a slow and moderate increase in pressure followed by a plateau lasting for hours (Fig. 1). On the second, and sometimes the third day, the injection had little pressor ef-