were present, they might have been lost during the recrystallizations. It is hoped to investigate this phenomenon further.

The above results are in qualitative agreement with the analyses of Harfenist & Craig (Table 1). The pig insulin differs from the cattle insulin in that an alanine and a valine residue are replaced by a threonine and an isoleucine residue respectively and the sheep insulin is the same as the cattle except that glycine replaces serine.

It is interesting that the only structural differences are in those residues which lie within the disulphide ring in insulin (Ryle *et al.* 1955). The fact that insulin is readily inactivated by any treatment that affects the disulphide bonds suggests that they may be concerned in its biological activity and it might seem that the part of the glycylic chain between positions A6-11, which contains three half-cystine residues, would be a physiologically important part of the molecule, perhaps the 'active centre'. However, it is clear from the present results that the activity of insulin is not dependent on the unique structure of the residues in these positions, since they can vary without any change in activity.

SUMMARY

1. The structures of pig and sheep insulin have been determined by subjecting them to partial hydrolysis and comparing the peptides produced with those obtained from cattle insulin.
2. The amino acid sequence in the phenylalanine chains is identical for all three insulins.
3. The glycylic chains are the same except for three residues in positions 8-10 inclusive. In the cattle

insulin this sequence is Ala.Ser.Val, in the pig insulin Thr.Ser.Ileu and in the sheep insulin Ala.Gly.Val.

Some of the experiments reported here were carried out by advanced students during a postgraduate course in biochemistry. We wish to express our gratitude to them and to Dr J. Lens of N. V. Organon, Dr J. G. Faber of N. V. Philips-Roxane, Dr O. K. Behrens of the Lilly Research Laboratories and Mr F. A. Robinson of Allen and Hanburys Ltd. for samples of pig and sheep insulin.

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Component Fatty Acids of Indian Snake Oils

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Pollard & McLaughlin (1950) reported that the lobes of snakes were the main depots of fat which is characterized by its higher content of unsaturated fatty acids. They also observed in the depot fat of the cottonmouth moccasin (*Agkistrodon piscivorus*) the presence of arachidonic acid to the extent of 11%. The rest of the fat was largely saturated, and hence it did not resemble marine animal oils or higher land animal fats, where higher unsaturated acids are predominant.

Tsujimoto & Kobayaschi (1920) proved the presence of C₂₀ and C₂₂ unsaturated acids by forming their ether-insoluble bromo derivatives from python

fat having an iodine value 80.3, while Gunstone & Paton (1953), using low temperature crystallization technique, confirmed that python fat was intermediate in character between higher land and marine animal fats.

In the present study additional work on the component fatty acids of Indian snake oils is reported.

EXPERIMENTAL

The snake oils from the python (*Python molurus*) and the diamond snake (*Python stinotis*) in the present study were kindly supplied to us by Dr J. N. Barowa of Victoria Gardens, Bombay. Both the oils were in a fresh state and

were yellowish in colour. These oils were saponified by the method of Hilditch (1947) and the mixed fatty acids were crystallized at different low temperatures from ether and acetone (10 ml./g.). Fractions having different iodine values were thus obtained (Table 1). Saturated fractions were methylated and the methyl esters were fractionated on an electrically heated packed column under reduced pressure (Longnecker, 1937).

Fraction *D*, obtained in the above crystallization, was a mixture of highly unsaturated fatty acids; it was therefore isomerized and the percentages of di- and tri-ethenoid acids were assayed (Hilditch, Patel & Riley, 1951) by measuring the absorption at 234 and 268 m μ . respectively with a Beckman spectrophotometer. The ultraviolet absorption spectrum of the isomerized sample was studied to investigate the presence of other polyunsaturated acids. An attempt

Table 1. *Low temperature crystallization of fatty acids from snake fats*

Python fat				Diamond snake			
Fraction	Weight		Iodine value	Fraction	Weight		Iodine value
	(g.)	(As % of total)			(g.)	(As % of total)	
A. Insoluble at -30° in ether (insoluble at -55° in acetone)	25.0	28.1	12.8	A'	25.1	29.8	30.5
*B. Soluble at -30° in ether (insoluble at -55° in acetone)	21.9	24.7	80.6	B'	34.1	40.5	64.4
*C. Insoluble at -70° in acetone (soluble at -55° in acetone)	22.0	24.9	80.8	C'	12.0	14.3	82.6
D. Soluble at -70° in acetone (soluble at -55° in acetone)	19.9	22.3	140.4	D'	13.0	15.4	190.7

* Fractions *B* and *C* from python fat were mixed and denoted as fraction *E* in Table 2.

Table 2. *Component acids of python fat*

All values except in last column are % (w/w) of total.

Acids	Fraction			Total	Moles %
	A	E	D		
Myristic	—	—	—	—	—
Palmitic	14.15	1.90	—	16.05	15.78
Stearic	8.26	1.84	—	10.10	9.80
Arachidic	1.75	0.50	—	2.25	1.99
Tetradecenoic	—	—	—	—	—
Hexadecenoic	0.33	4.47	—	4.80	5.21
Octadecenoic	2.37	38.90	—	41.27	45.24
Octadecadienoic	—	0.27	7.33	7.60	7.47
Octadecatrenoic	—	—	3.00	3.00	2.97
Eicosenoic	1.24	1.70	8.93	11.87	10.54
Docosenoic	—	—	3.06	3.06	2.49

Table 3. *Component acids of diamond snake oil*

All values except in last column are % (w/w) of total.

Acids	Fraction				Total	Moles %
	A	B	C	D		
Myristic	0.39	0.86	0.35	—	1.60	1.95
Palmitic	11.17	3.57	1.01	—	15.74	17.01
Stearic	7.82	5.40	0.08	—	13.30	13.09
Arachidic	1.03	—	—	—	1.03	0.84
Tetradecenoic	0.01	0.07	0.12	—	0.20	0.25
Hexadecenoic	1.73	2.56	0.88	—	5.17	5.6
Octadecenoic	5.80	22.84	8.12	—	36.76	36.21
Octadecadienoic	—	0.08	0.67	8.69	9.44	9.5
Octadecatrenoic	—	—	—	3.16	3.16	3.2
Eicosenoic	1.85	5.14	3.02	2.78	12.79	11.5
Eicosatetraenoic	—	—	—	0.81	0.81	0.85

was also made to prepare the bromo derivatives (Markley, 1947) of this fraction.

The composition of the whole oil was calculated from the iodine values and saponification equivalent of each individual fraction obtained after methyl ester fractionation (Hilditch, 1947) (Tables 2 and 3).

RESULTS AND DISCUSSION

Python fat had iodine value 75.4 and saponification equivalent 292.9; its mixed fatty acids showed iodine value 75.7.

In diamond snake oil, the iodine value and saponification equivalent observed were 76.7 and 289.9 respectively, and the mixed fatty acids had iodine value 76.8.

Saturated acids were present in both the oils, almost to the same extent; but in diamond snake oil the percentage was slightly higher. Palmitic and stearic acids were the major components.

Though diamond snake oil contained much hexadecenoic and eicosenoic acids, python fat had a definitely higher content of octadecenoic acid (41%). More than 10% eicosenoic acid was found in both fats and it seemed to be characteristic of these fats. Octadecadienoic and octadecatrienoic acids were present in both, almost to the same extent.

Higher polyunsaturated acids were present only in traces. Eicosatetraenoic acid was present (0.81%) in diamond snake oil, but was absent from python fat. Docosenoic acid (3%), however, was found in python fat. The spectra shown by fractions *D* and *D'* of python fat and diamond snake oil after isomerization with 7.5% alkaline ethylene glycol at 180° for 1 hr. and at 170° for 15 min. indicated the presence of 33.7 and 56.3% of octadecatrienoic acid and 13.5 and 20.5% of octadecadienoic acid respectively. The absorption spectrum of the isomerized sample of fraction *D* of diamond snake oil showed a low peak at 303 m μ . and a failure to isolate an ether-insoluble bromo derivative indicated that eicosatetraenoic acid occurred only in traces.

Fractions *D* and *D'* were highly coloured and had saponification equivalents 309 and 320 (approximately) respectively. Apart from a small amount of eicosatetraenoic acid in one case, the polyethenoic acids responsible for the ultraviolet absorption after isomerization were assumed to belong to the C_{18} series only. From these absorption values and the iodine value of the fraction (*D* or *D'*), it was possible to calculate the iodine value of the remaining acids, which from the high saponification equivalents must be mainly from the C_{20} and C_{22} series.

Comparison of the present data with those for python fat obtained by Gunstone & Paton (1953) shows that the iodine values of all these fats were very close (Table 4). The saturated acid content was

almost the same in all cases. The python fat analysed by the British authors contained more palmitic acid than the two Indian snake oils. There was, however, no marked difference in the stearic acid content.

Table 4. *Component acids* (% w/w) *of python fat and diamond snake fat*

Acids	Python fat (present work)	Diamond snake fat (present work)	Python fat (Gunstone & Paton, 1953)
Myristic	—	1.60	1.3
Palmitic	16.05	15.74	19.7
Stearic	10.10	13.30	10.8
Arachidic	2.25	1.03	1.2
Tetradecenoic	—	0.20	0.5
Hexadecenoic	4.80	5.17	3.9
Octadecenoic	41.27	36.76	47.0
Octadecadienoic	7.60	9.44	10.7
Octadecatrienoic	3.00	3.16	0.8
Eicosenoic	11.87	12.79	4.1
Eicosatetraenoic	—	0.81	—
Docosenoic	3.06	—	—

Among the unsaturated acids of python fat (Gunstone & Paton, 1953) octadecenoic acid was a major component, followed by octadecadienoic acid, eicosenoic acid being present in small amount. In the Indian snake oils, octadecenoic acid was predominant; it was next followed by eicosenoic acid, whilst octadecatrienoic acid was present only in traces.

Both the polyunsaturated acids found by us (eicosotetraenoic acid in diamond snake oil, and docosenoic acid in python fat) were completely absent from the fat analysed by Gunstone & Paton (1953).

The presence, in snake oils, of unsaturated acids such as octadecadienoic, octadecatrienoic, eicosenoic and even eicosatetraenoic and docosenoic acids provides a striking difference from the depot fats of higher land animals. The content of polyunsaturated fatty acids was much smaller than in a typical marine animal oil. Hence it may be concluded that snake fats are typical neither of higher land animal fats nor of marine animal oils, but form a link between the two as suggested by Klenk (Klenk, 1933; Klenk, Ditt & Diebold, 1935) and by Hilditch (1947).

SUMMARY

1. The component fatty acids of the oils of two Indian snakes (*Python molurus* and *P. stinotis*) were studied using low temperature crystallization technique.

2. Palmitic and stearic acids were the major components present in the saturated portion.

3. Among the unsaturated acids, octadecenoic acid was present together with eicosenoic acid.

4. Polyunsaturated acids were absent, eicosatetraenoic acid was present only in traces in diamond snake oil but docosenoic acid occurred to the extent of 3 % in python fat.

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Liver Catalase in the Riboflavin-deficient Mouse

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Work so far carried out in this laboratory has shown that testicular and adrenal hormones and trace metals are of importance in the maintenance of liver-catalase activity in the normal mouse (Adams, 1950*a*, 1952, 1953*a*). In the search for additional factors influencing the catalase activity the effects of riboflavin deficiency have been studied, and some results of this work are described in the present paper. It had been hoped to pursue the subject further before publication, but work has been held up for some time by an infection of the liver in the strain of mice used ('Tyzzer's disease'). Previous experience here has indicated that a variety of infectious diseases in which the liver is directly involved ('Tyzzer's disease', salmonella infections, and ectromelia) result in a lowered liver-catalase activity, and a reduction or disappearance of the normal sex difference in enzyme level. Further, under such conditions, attempts to change the liver catalase level by hormone infection are quite unsuccessful. Since two brief reports of the present work on riboflavin deficiency have already appeared (Adams, 1953*b*, 1954) it seemed desirable to publish in full the experimental results obtained up to the outbreak of the epidemic. These results suggest that testosterone, which is responsible for the sex difference in catalase activity in normal mice, requires the presence of riboflavin for its action on the enzyme level.

EXPERIMENTAL

Animals. Mice of a stock albino strain were used. Water was provided *ad lib*.

Diets. Mice fed normal diet were given commercial 'rat'

cubes prepared according to the Rowett Institute formula (Thomson, 1936).

A diet designed to induce riboflavin deficiency was prepared by British Drug Houses Ltd. according to the following recipe:

	%
Casein, 'fat and vitamin free'	21
Sucrose	32.5
Rice starch	32.5
Arachis oil	9.1
Agar	1.0
Salt mixture	2.0
Succinyl sulphathiazole*	1.0
Vitamin mixture	0.85

* Altered to succinyl sulphathiazole, 0.75 %, streptomycin sulphate, 0.25 %, in later experiments.

The salt mixture was prepared according to the method of Hubbel, Mendel & Wakeman (1937).

The vitamin mixture per 5 kg. of diet was as follows: thiamine 30 mg., pyridoxine 30 mg., biotin 1 mg., nicotinic acid 250 mg., calcium pantothenate 200 mg., folic acid 17 mg., inositol 200 mg., *p*-aminobenzoic acid 100 mg., vitamin B₁₂ 1 mg., ascorbic acid 500 mg., tocopherol acetate 350 mg., 2-methyl-1:4-naphthoquinone (Menaphthone) 150 mg., choline chloride 20 g., cod liver oil 20 g. These substances were mixed in with the rest of the diet. Since the average amount of diet eaten by the mice was about 3 g. each/day, each mouse received approximately 1/1700 of these quantities/day. The powder was moistened with sufficient water to give a rather dry dough (approximately 1 ml./5 g. of diet). The diet of pair-fed control groups receiving a riboflavin supplement was prepared by moistening the powder with water containing 25 µg. of riboflavin/ml. (i.e. providing 15 µg./mouse/day).

Mice were placed on the diets at weanling age (weight about 12 g.).