[2(ring)-^{14}C]histidine. Labelled ergothioneine was isolated from these cultures and chemically degraded.

2. The intact imidazole ring and side chain of histidine were shown to be incorporated into ergothioneine by this fungus.

The authors wish to thank Professor C. Rimington, F.R.S., for his interest in this problem and Mr P. R. E. Wallace and Mr L. Bowler for technical assistance. Burroughs Wellcome Ltd. generously provided us with natural ergothioneine.

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The Inhibition by γ-Irradiation of Incorporation of ^{32}P into Rat Thymocytes *in vitro*

By MARGERY G. ORD and L. A. STOCKEN
Department of Biochemistry, University of Oxford

(*Received* 29 *July* 1957)

In a search for the primary biochemical lesions which are produced by ionizing radiation in animals, it would be convenient to have a mammalian system which is radiosensitive *in vitro* and which would provide sufficient material to permit a biochemical investigation. The inhibition of uptake of precursor into the nucleic acids of different tissues is a well-known consequence of irradiation *in vivo* (Euler & Hevesy, 1942) and can be detected very soon after the end of exposure (see Mitchell, 1956; Ord & Stocken, 1957). Howard & Pelc (1951), Lajtha, Oliver & Ellis (1954) and Totter (1954) have demonstrated the radiosensitivity of bean-root tips and bone-marrow cultures and suspensions to radiation *in vitro*, the inhibition of precursor uptake into nucleic acids being used as an index of radiation damage. Trowell (1952) found that cultures of lymph node are sensitive to exposure *in vitro*, and by using his cultures we have shown that incorporation of ^{32}P into their deoxyribonucleic acid is affected before histological damage can be detected (Ord & Stocken, 1956). On the other hand, the experiments on uptake of ^{32}P into the nucleic acids of thymus suspensions after exposure to 1000–2000 r. of γ-irradiation *in vitro* gave equivocal results (Ord & Stocken, 1956). The development of a medium suitable for the isolation of nuclei (Barnes, Esnouf & Stocken, 1957) prompted a re-examination of the behaviour of thymus suspensions in this medium, and they have now been found to be radiosensitive. It has therefore been possible to investigate some of the biochemical changes which accompany the inhibition of metabolism of thymus deoxyribonucleic acid.

METHODS

Animals. Young animals (70–130 g.) of the Laboratory stock of Wistar rats were used throughout. The results appeared to be independent of the sex and weight of the animal within this range.

Thymus suspensions. In the earlier experiments thymi from several rats were pressed through a stainless-steel gauze, suspended in a potassium-rich medium (0·085M-KCl, 0·0085M-NaCl, 0·0025M-$CaCl_2$, 0·0025M-$MgCl_2$, 0·005M-triethanolamine hydrochloric acid buffer, pH 7·2) supplemented with glucose (final concentration 10 mg./ml.) (Barnes *et al.* 1957) and filtered without squeezing through a single layer of muslin. This produced suspensions with not more than 30% of whole cells. To obtain 80–90% of undamaged cells, the capsule at the base of the thymus was sliced and the contents of the gland were gently scraped free. The operation was performed on a glass surface cooled with ice, and the resulting material was suspended in medium. From three to four rats 1 g. (wet wt.) of extruded thymocytes free from connective tissue was obtained, suspended in 4 ml. of medium and filtered as before. For irradiation and incorporation of ^{32}P, 1 ml. of suspension was incubated in Warburg flasks at 37°. It was thus possible to irradiate the cells while they were actively respiring and to measure the oxygen consumption during the experiment.

Cell counts. These were very kindly performed by Mr M. P. Esnouf by the differential-phase-contrast technique of Barer, Joseph & Esnouf (1956). Whole cells were distinguished from damaged and pycnotic cells and from nuclei. The results are given as total nuclei/ml. of suspension and as percentage of normal cells. Sufficient cells were counted to give an accuracy of ±5%.

Irradiation. Irradiation was provided by two 250 mc radium sources which were placed in Perspex holders beneath Warburg flasks (5 ml. capacity) containing 1 ml. of suspension (Ord & Stocken, 1955). With one source

beneath each vessel, a dose of 950 r. was received in 30 min. The dose was estimated by the ferrous sulphate-oxidation method (Miller, 1950).

Incorporation of ^{32}P. Carrier-free ^{32}P was obtained from the Radiochemical Centre, Amersham, Bucks. Unless otherwise stated, 25 μc was used/ml. of thymocyte suspension. In some experiments ^{32}P was given intramuscularly to the rats some hours before death, so that the release of ^{32}P from the organophosphates of thymus *in vitro* might be followed.

Inorganic phosphate. This was estimated by the method of Berenblum & Chain (1938).

Acid-soluble nucleotides. After incubation the flasks were chilled in ice and the protein was precipitated by 0·1 ml. of 100% (w/v) trichloroacetic acid. The suspensions were centrifuged and the supernatants stored at −20° until required. The acid-soluble nucleotides were separated by paper chromatography by the technique of Krebs & Hems (1953), which allows removal of the inorganic phosphate. Usually 7 μl. of the supernatant was taken, but in some experiments samples from the concentrate obtained by evaporation from the frozen state were used. Inosine triphosphate, guanosine triphosphate, guanosine diphosphate, uridine triphosphate, uridine diphosphate, adenosine diphosphate (ADP) and chromatographically pure adenosine triphosphate (ATP) (Sigma Chemical Co., St Louis, Mo., U.S.A.) were used as markers. The nucleotides were located by ultraviolet photography.

Nucleic acid extraction. Deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and deoxyribonucleotides were separated from the trichloroacetic acid precipitate as previously described (Deluca, Rossiter & Strickland, 1953; Richmond, Ord & Stocken, 1957). Our earlier experiments had demonstrated that incorporation of ^{32}P into DNA isolated by this technique reflected uptake of isotope into the deoxyribonucleotides. Blanks were run with thymus suspensions to which trichloroacetic acid was added before incubation with ^{32}P. Uptake into DNA under these conditions was negligible.

Measurement of radioactivity. ^{32}P activity in the inorganic phosphate, nucleic acids and nucleotides was measured in a Geiger–Müller liquid counter.

The location of the acid-soluble nucleotides on the chromatogram was detected by a radioautograph. The activity/unit area of paper was then measured by an end-window scanner. Sufficient counts were recorded to give an error of less than 3%.

RESULTS

Incorporation of ^{32}P *into nucleic acids.* In our earlier experiments it was suggested that the equivocal response to γ-irradiation shown by the rat-thymus preparations *in vitro* was due to structural damage to the cells which was detectable very shortly after their isolation (Ord & Stocken, 1956). The thymus suspensions in the high-potassium medium (Barnes *et al.* 1957) were therefore examined by phase-contrast microscopy initially and after 1 and 2 hr. of incubation at 37°. The appearance of the cells in the medium seemed normal and very little increase in pycnosis occurred during shaking for 2 hr. in the Warburg apparatus at 37°. Motility, however, was not used as an index of cell integrity.

In the first experiments (Table 1, group A) the suspensions were supplemented with 20 μmoles of succinate and 1 μmole of ATP, and were gassed for 2–3 min. with O_2 before incubation. In the first two experiments of the group, ^{32}P was incubated with the suspensions during the period of irradiation (30 or 60 min.) and the reaction was stopped as soon as the dose had been delivered. Since it may be presumed that incorporation starts with the addition of the isotope it is clear that a significant uptake will have occurred before irradiation can have produced a measurable effect. It was therefore decided for all subsequent experiments to irradiate the samples and to tip in the tracer as soon as the dose had been delivered. If both sources were present under one Warburg flask 950 r. were received in 15 min., and the procedure was therefore adopted of setting up paired vessels at 37°, one of which was irradiated. After 15 min. the sources were removed and ^{32}P was tipped into both suspensions. The incubations then continued for a further 30 min. period. It can be seen (Table 1, groups B and C) that the degree of inhibition depended on the dose received.

It was also noticed in the first group of experiments that if, under comparable conditions, the number of whole cells in the preparation was increased, uptake of ^{32}P into the nucleic acids also increased although the sensitivity of the incorporation was unaffected. This suggested that in this medium, uptake of precursor into DNA occurred only in the intact cells. This was supported by an experiment (Table 2) in which nuclei were prepared (Barnes *et al.* 1957) from a minced suspension, and uptake of ^{32}P into DNA and nuclear RNA of these nuclei was compared with that into DNA and RNA of a preparation of whole cells. Negligible incorporation occurred into DNA of the nuclei, although some uptake took place into nuclear RNA. This latter finding requires confirmation.

Since the sensitivity of the preparations in this first group of experiments was not great, it was decided to investigate the possibility of a protective effect due to the presence of the substrate (Dale, 1940). When succinate and ATP were omitted (Table 1, group B) it can be seen that uptake into DNA was reduced even more by irradiation, whereas that into RNA remained virtually unaffected. It was also established for a control suspension (Table 2) that the adjuvants did not alter the relative uptakes into RNA and DNA; all subsequent experiments were therefore performed without additions. Oxygen uptakes were always measured during the last 15 min. of incubation. Omission of succinate and ATP naturally reduced O_2 uptake, but in the large

Table 1. *Effect of γ-irradiation on the incorporation of ^{32}P into nucleic acids of rat-thymus suspensions*

Gas phase was oxygen unless otherwise indicated in parentheses in the first column. Suspensions of group A contained succinate and ATP (20 μmoles and 1 μmole respectively); those of groups B and C had no adjuvants. Dose: groups A and B, 950 r.; group C, 600 r.

	Cell counts		Specific activity (counts/min./μg. of P)				
			DNA		RNA	RNA/DNA	
	Total nuclei present (10^{-8} × no./ml.)	Percentage of normal cells	Control	Irradiated as % of control	Irradiated as % of control	Control	Irradiated
Group A	3·09	34	2·95	90*	110	8·15	9·93
	2·62	17	5·66	76·5†	115	7·35	11·1
	4·2	35	1·67	81	128	3·82	6·04
	5·02	88	12·3	85	107	3·55	4·47
	4·29	87	11·7	88	86	3·46	3·36
Mean (last three expts.)	4·50	—	—	85	107	3·61	4·82
Group B	4·35	84	4·34	64	99	3·1	4·8
	4·34	78	5·12	60·5	95	2·97	4·66
	3·51	78	3·02	81	98	4·62	5·60
	4·78	81	2·44	83	97	5·43	6·33
	3·61	87	3·7	64·5	93	3·65	5·25
	6·56	90	3·91	66	105	4·63	7·37
	4·3	83	2·46	80	102	4·21	5·39
	7·3	91	4·90	56·5	106	2·62	4·9
	7·1	91	4·51	78·5	125	2·54	4·05
	4·98	77	5·63	79·5	96	2·66	3·2
	4·18	87	6·49	74·5	106	2·5	3·57
	6·24	83	7·93	64	91	2·45	3·49
Mean	5·1	84	—	71	101	3·45	4·88
Group C	5·34	88·5	1·33	88	84	3·57	3·48
(Air)	5·34	88·5	1·69	83	100	3·54	4·26
	4·74	82	1·46	87	98	3·8	4·3
(Air)	4·74	82	1·97	91·5	91	3·8	3·98
	4·13	83·5	2·49	83·5	99	2·6	3·0
(Air)	4·13	83·5	3·36	84	120	2·56	3·68
Mean	4·74	85	—	86	99	3·31	3·78

* In the presence of ^{32}P (see text). † Dose 1900 r., in the presence of ^{32}P.

Table 2. *Incorporation of ^{32}P into nucleic acids of rat-thymus suspensions under different experimental conditions*

Details of these experiments are given in the text. The concentrations of succinate and ATP were as in Table 1.

	Cell count			Specific activity (counts/min./μg. of P)		
	Total nuclei (10^{-8} × no./ml.)	Percentage of whole cells	Uptake of O_2 (μl./15 min.)	DNA	RNA	RNA/DNA
Cells	3·96	80	31·6	11·1	25·0	2·25
Nuclei	5·17	10	5·0	0·67	9·33	—
Cells						
(*a*) With succinate and ATP	5·28	80	63	9·09	44·5	4·9
(*b*) Without succinate and ATP	5·28	80	46·6	1·92	9·8	5·1

number of experiments performed under standardized conditions without adjuvants, O_2 uptake was proportional to the total number of nuclei present in the preparation (whole cells, damaged cells, nuclei and free cytoplasm), and the O_2 uptakes did not differ significantly between the control and irradiated suspensions.

The last group (C) of experiments shown in Table 1 tested the effect of O_2 on the radiosensitivity of the preparation. Increased sensitivity in the presence of O_2 is a well-established factor in radiobiology (Gray, Conger, Ebert, Hornsey & Scott, 1953; Laser, 1956). Since anaerobiosis prevents the incorporation of ^{32}P into DNA (Findlay, Rossiter & Strickland, 1953) it was only practicable to compare the effects of irradiation on samples which had either O_2 or air as the gas phase during the experimental period. It can be seen (Table 1, group C) that the alteration in O_2 tension did not increase the sensitivity of the thymocytes but that flushing with O_2 appeared to reduce the incorporation of ^{32}P into both DNA and RNA; gassing was therefore discontinued in later experiments.

In experiments on the uptake of precursors into nucleic acids *in vitro* it was observed (Thompson, Smellie, Goutier & Davidson, 1956) that the ratio of incorporation into purine and pyrimidine deoxyribonucleotides was much less than that found *in vivo*. This was attributed to failure of the cells to synthesize purines at a sufficient rate, and it has been suggested (Davidson, Thompson, Paul, Smellie & Goutier, 1957) that it might be overcome in the intact rat by supplies from the liver. In our experiments, although a decreased uptake of ^{32}P into purine deoxyribonucleotides relative to values obtained *in vivo* (Ord & Stocken, 1956) was very evident (Table 3), irradiation still reduced the incorporation into all four deoxyribonucleotides.

Analysis of acid-soluble phosphates. The results shown in Table 1 indicated that γ-irradiation *in vitro* blocked uptake of ^{32}P into thymus DNA without affecting that into RNA. Perhaps the simplest explanation might be dilution of the inorganic phosphate pool in the irradiated samples by release of phosphorus from non-labelled compounds. This was investigated with suspensions of thymus which had been labelled with ^{32}P *in vivo*. An interpretation of the results of these leakage experiments was simplified by the absence of phosphate from the suspension medium. The concentration and specific activity of inorganic phosphate in trichloroacetic acid supernatants from these thymus suspensions after irradiation *in vitro* showed no difference in levels of either ^{31}P or ^{32}P between control and exposed preparations (Table 4). In the two experiments in which thymus nuclei were prepared and examined there was also no evidence for leakage of inorganic phosphate. This was confirmed in experiments (Table 5) in which uptake of ^{32}P again occurred *in vitro* after irradiation and the concentration and specific activity of the inorganic phosphate were measured, as well as the specific activity of the nucleic acids. Chromatographic separation of the acid-soluble nucleotides of the supernatant was also performed, and we are indebted to Dr G. V. R. Born for the estimation of the levels of ATP in the extracts by the firefly method (Strehler & Totter, 1952). It will be seen that again there were no consistent differences between the inorganic phosphate levels and specific activities of control and irradiated samples.

The chromatograms were run either with supernatants, used directly with ATP and other nucleotides as tracers, or with preparations which had been concentrated by evaporation from the frozen state. In the first method there was insufficient material in the absence of tracer to indicate the presence of the nucleotides by ultraviolet absorption, but the labelled components could easily be detected by radioautography. In the second method it was possible to demonstrate directly the presence of two main nucleotide components in the incubated samples, but neither method showed any qualitative differences between the components from control and irradiated suspensions. The faster-moving nucleotide was identified as ATP from its R_F and from its behaviour with tracer ATP. Only small amounts of ADP were detected in incubated samples and negligible activity was associated with it. The specific activity and amount of ATP was measured in preliminary experiments by elution of the nucleotide region on the chromatograms run with concentrated material. Since the amounts of ATP were low and the phosphorus blank was high, although the results showed a very marked increase in activity of the irradiated samples, an alternative method was finally adopted of locating by radioautograph the activity corresponding to ATP on the chromatograms, and then equal areas from

Table 3. *Relative specific activities of thymus deoxyribonucleotides* in vivo *and* in vitro

	Adenine	Guanine	Cytosine	Thymine
In vivo	0·71	0·93	0·57	1·0
In vitro	0·08	0·13	0·92	1·0
Effect of exposure to 950 r. *in vitro* (as % of control)	80	66	81	74

Table 4. *Effects of* 950 *r. of γ-irradiation on the release of inorganic phosphorus from thymus suspensions labelled* in vivo

Cell count		Phosphorus leak 15 min. after end of irradiation			
		^{31}P (μg./ml. of supernatant)		^{32}P (counts/min./μg. of P)	
Total nuclei (10^{-6} × no./ml.)	Percentage of whole cells	Control	Irradiated	Control	Irradiated
0·204	6·6	4·7	4·2	52	52
0·202	4·1	3·4	2·8	71	60
3·88	80	36·3	36·0	—	—
4·41	80	39·8	39·6	—	—
5·16	83·3	44·0	41·0	22·4	24·5
6·19	84·1	33·9	35·0	25·1	25·1
4·7	85·2	34·6	39·4	22·2	18·8

Table 5. *Concentration and specific activity of acid-soluble phosphates of thymus suspensions after* 950 *r. of γ-irradiation* in vitro

Concentrations are expressed as μg. of P/ml. and specific activities (S.A.) as counts/min./μg. of P. C, Control; R, irradiated suspension.

	Inorganic phosphate				ATP			Guanosine triphosphate S.A.	DNA S.A.		RNA S.A.	
	Concn.		10^{-4} S.A.		Concn.							
				100R		100R	S.A. 100R	100R		100R		100R
	C	R	C	C	C	C	100R/C	C	C	C	C	C
	33·9	35·0	9·3	98	—	—	138	—	3·52	66	16·3	105
	28·0	31·8	14·7	90	—	—	125	108·5	5·75	78·5	14·6	125
	29·9	29·6	14·3	101	—	—	100·5	105·5	6·0	56·5	15·7	106
	32·3	31·1	12·4	98	—	—	122	105·5	3·78	79·6	15·9	102
	27·6	30	12·2	93·5	11·1	96·5	101	100	5·63	79·5	14·95	96
	30·1	28·1	11·2	108	12·0	110	113·5	108	6·49	74·5	16·2	106
	34·8	35·6	9·5	100	13·2	108	107	99	7·95	64	19·4	91
Mean	30·9	31·6	—	98	—	105	115	104	—	71	—	104

control and irradiated suspensions were counted with an end-window scanner. For this purpose the trichloroacetic acid supernatants were not concentrated, and equal amounts were taken from each sample. The samples were run at least in duplicate. It can be seen that there was an increase in ^{32}P activity of ATP from the irradiated suspensions.

The other nucleotide on the chromatograms had a considerably smaller R_F than ATP, and intervening between these regions there were at least two major components which were not nucleotides. These were thought to be sugar phosphates but were not further examined. The slower-moving nucleotide was identified as guanosine triphosphate from its ultraviolet absorption (Beaven, Holiday & Johnson, 1955), and from its behaviour on chromatograms run with guanosine tri- and di-phosphate as tracers.

DISCUSSION

In these experiments it has been possible to obtain reproducible inhibition of incorporation of ^{32}P into the DNA of rat-thymus suspensions by irradiation *in vitro*. We have not investigated in detail media other than the one used here, so that it is not possible to say whether these effects might also be produced with nuclei rather than with whole cells. Allfrey, Mirsky & Osawa (1955, 1957) have shown, in a sucrose medium, that isolated thymus nuclei are metabolically active especially in protein synthesis and the intranuclear generation of ATP. They also reported that other media tried were not suitable for demonstrating this activity, which may explain the failure of thymus nuclei in our experiments to incorporate ^{32}P.

The radiosensitivity of the incorporation was decreased by the presence of succinate and ATP in the medium, and experiments demonstrated that the ATP was very rapidly hydrolysed, probably to the adenosine monophosphate level. Protection of cell suspensions against irradiation by oxidizable substrates is well established, particularly with micro-organisms (cf. Goucher, Kamei & Kocholaty, 1956).

Lajtha, Oliver, Kumatori & Ellis (1958) have shown with bone-marrow preparations *in vitro* that the synthetic period (*S*) in interphase is less sensitive than the G_1 period immediately preceding it. They concluded that 'There is a system connected with, but not identical to, DNA synthesis which is more sensitive than the process of synthesis itself.' They did not investigate RNA or

other cytoplasmic components in any detail. Since ^{32}P was incubated with our suspensions for only 30 min. immediately after exposure, if the timing of the mitotic cycle in thymocytes is comparable with that in bone marrow then the effects on DNA must have been predominantly with cells in the *S* period. Their sensitivity appears to be similar to that reported by Lajtha *et al.* (1958).

The lack of effect on RNA resembles results obtained for incorporation of ^{32}P with lymph-node cultures (Ord & Stocken, 1956), where inhibition of uptake of ^{32}P into DNA was obtained 2 hr. after exposure to 600 r. *in vitro*, with no effect on RNA and no histological signs of damage to the cells. In thymus, by 30 min. after 1000 r. of total body irradiation, nuclear and cytoplasmic RNA are affected as well as DNA (Ord & Stocken, 1957), but in liver, although ^{32}P uptake into DNA is reduced, that into RNA is unaffected or increased (Sherman & Almeida, 1957; Richmond *et al.* 1957). It is not yet possible to say whether the effect on RNA metabolism *in vivo* occurs as a result of damage to the DNA system, the whole process proceeding more rapidly than with isolated preparations, or whether the suspensions and cultures used *in vitro* may have lost that part of the radiosensitive mechanism which causes reduction in RNA metabolism *in vivo*. A further consideration is that isolated preparations are not exposed to secondary changes which occur in the intact animal as a result of exposure (Holmes, 1949; Harrington & Lavik, 1957). Whatever the explanation for the results *in vivo* it is clear that in these experiments *in vitro* incorporation of ^{32}P into DNA was reduced without alteration of RNA, and this was accompanied by an increased turnover of ^{32}P into ATP. Mitchell (1942) reported the accumulation of a nucleotide-like material in the cytoplasm of cells after irradiation and suggested that exposure produced a block between RNA and DNA. Sherman & Forssberg (1954) and Forssberg & Klein (1954) found transient alterations in the levels and specific activities of adenine polynucleotides in liver and ascites-tumour cells immediately after exposure *in vivo*; in mouse liver the specific activity of ATP was increased 5 min. after exposure. In our experiments with rat thymus we could detect no change in incorporation of ^{32}P into acid-soluble nucleotides 1 and 2 hr. after exposure to 1000 r. *in vivo*, but the nucleotides were estimated by chemical means and not by the more accurate chromatographic separation. Since in the experiments *in vitro* uptake of ^{32}P into RNA was not inhibited, it appears either that the machinery for synthesis of ribonucleotides is unaffected by γ-irradiation or that there is an adequate reserve of utilizable precursors for the duration of the experiment. The increase in specific activity of ATP suggests either that the ATP is not available to the nucleus for DNA synthesis or that there is block in the generation of deoxyribonucleotides (cf. Mitchell, 1942). It may be significant that enucleation in amoebae causes an accumulation of ATP in the cytoplasm under aerobic conditions (Brachet, 1955, 1956).

SUMMARY

1. A medium has been found in which a large proportion of a respiring suspension of thymus cells retain their normal appearance, as judged by phase-contrast microscopy, for periods up to 2 hr.

2. The uptake of ^{32}P into the deoxyribonucleic acid of the respiring thymocytes was inhibited by 600–950 r. of γ-irradiation.

3. There was an increase in the specific activity of adenosine triphosphate in the irradiated suspensions.

4. Incorporation of ^{32}P into ribonucleic acid was unaffected *in vitro*.

We are indebted to the late Lord Cherwell for permitting the loan of the radium sources. We are also extremely grateful to Messrs L. V. Eggleston and R. Hems for considerable advice on nucleotide chromatography and to Dr G. V. R. Born and Mr M. P. Esnouf for measuring the ATP and cell contents of our suspension. Mr J. A. Morris gave us skilled and untiring technical assistance.

This work was aided by grants to the Department from the Rockefeller Foundation and the Nuffield Foundation for Medical Research.

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The Chemical Composition of Earthworm Cuticle

By M. R. WATSON
Medical Research Council Biophysics Research Unit, King's College, Strand, W.C. 2

(*Received* 5 *July* 1957)

Since the work of Astbury (1938) and Bear (1952) it has become usual to define collagen as a group of fibrous proteins which give a characteristic wide-angle X-ray-diffraction pattern, and collagens defined in this sense have been found widely distributed throughout the animal kingdom (Bear, 1952; Rudall, 1955). That a large number of different proteins have identity of wide-angle X-ray-diffraction pattern implies that these proteins have certain features of their molecular structure in common, and the present definition therefore includes all those proteins in which these features are present.

The highly characteristic amino acid composition of collagens derived from mammalian sources, and especially the fact that in many analyses the glycyl residues appear to be exactly one-third of the total number of residues present, has provoked much comment, and indeed in some models is a prerequisite of the stereochemical packing of the polypeptide chains. It is, in this context, interesting to explore the range of chemical composition which is possible within the framework of the collagen structure. Mammalian collagens form a compact group with regard to their amino acid compositions (Eastoe, 1955; Neuman, 1949), and therefore for this purpose it is necessary to examine collagens from a wider range of animals.

Earthworm cuticle has been shown to give the wide-angle X-ray pattern of collagen (Astbury, 1938; Rudall, 1950) and is readily obtained in a morphologically pure state for chemical analysis. A preliminary account of this work has been published (Watson & Smith, 1956).

EXPERIMENTAL

Isolation of earthworm cuticles. Batches of 100 earthworms (*Lumbricus* sp.) were killed by drowning them in ether. After immersion for 15 min. the cuticles may be readily removed by rolling them back from the anterior end with forceps. The cuticles were then washed with numerous changes of water in order to remove adhering columnar epithelial cells. In the first two preparations this was followed by incubation with 0·1% trypsin in tap water at room temperature for 8 hr. The cuticles were finally washed in water. For the determination of the hydrothermal-shrinkage temperature the cuticles were used without further treatment. For chemical analysis the cuticles were dehydrated with two changes of absolute ethanol and two changes of ether, and were allowed to dry in air. At this point the cuticles should appear quite white and any yellow coloration may be taken as an indication of the presence of epithelial material, in which case the washings should be repeated. The yield of air-dry cuticle was usually about 300 mg. The cuticles were then cut into small pieces with scissors, and mixed thoroughly. The samples were allowed to equilibrate for 48 hr. with the air of the laboratory before weighing.