The Effects of X-Irradiation on the Incorporation of Amino Acids into Proteins of Nuclei from Rat Thymus Gland

By J. A. SMIT AND L. A. STOCKEN

Department of Biochemistry, University of Oxford

(Received 13 August 1963)

There are inconsistencies in the reports of experiments on the effects of whole-body irradiation on protein synthesis. Some workers observed increases in synthetic activity soon after irradiation (Hevesy, 1949; Richmond, Altman & Salomon, 1951; Kay & Entenman, 1956; Butler, Cohn & Crathorn, 1957), others noticed no change (Abrams, 1951; Holmes & Mee, 1952; Killander, Ribbing, Ringertz & Richards, 1962) and some (Hempelmann, Carr, Frantz, Masters & Lamdin, 1950; Richmond, Ord & Stocken, 1957; Salganik, 1958) reported a decrease after irradiation. These erratic reports, especially the observed stimulation of synthesis, can be explained by the increased secretion of adrenocorticotrophic hormone from the pituitary gland after subjection to stresses (Selye, 1957; Bransome & Reddy, 1963) such as that caused by irradiation. This leads to the secretion from the adrenal cortex into the blood of abnormally large quantities of steroid hormones (glucocorticoids) that affect the metabolism of carbohydrates, proteins and lipids (Bacq & Alexander, 1961). The release of adrenaline after radiation has also been shown to have a radioprotective effect as a result of its antioxidant activity (Bekkum & Cohen, 1955). It seems that much could be gained, in a study of the fundamental effects caused by irradiation *in vivo*, if the adrenal glands were first removed. The present paper describes differences in response to whole-body X-irradiation of normal and adrenalectomized rats as shown in the rates of protein synthesis in nuclei prepared from the thymus gland. It also shows that the ability of nuclear enzymes to synthesize proteins depends on free thiol groups. A preliminary account of these findings has already been reported (Smit & Stocken, 1963a).

METHODS

Animals. Animals weighing 100–130 g. (4–5 weeks old) were taken from the laboratory stock of Wistar rats. In most experiments females that had been kept from weaning in a room with a 12 hr. light period, from 8 a.m. to 8 p.m., were employed.

Isolation of nuclei. The thymus glands used in the preparation of nuclei were removed from the rats about 1 min. after decapitation for experiments *in vitro*. For experiments *in vivo*, with groups of animals, the removal time was

carefully controlled with a stop-watch, the glands were removed with forceps within 2 sec. of the time stated and placed in semi-frozen medium. Nuclei were prepared in sucrose medium [sucrose (0.25 M) and CaCl_2 (3.3 mM) in 5 mM-tris-HCl buffer, pH 7.3 at 20° (glass electrode)] by the method of Creasey & Stocken (1959). The final concentration of the nuclear suspension was usually the equivalent of one thymus/ml. of medium.

Incubation conditions for amino acid incorporation. The incubation mixture consisted of: nuclear suspension (1.0 ml.); sucrose medium (see above) (0.1 ml.) containing 12 μC of DL-[G- ^3H]valine (specific activity 480 mc/m-mole), glucose (240 μmoles) and NaCl (240 μmoles); and sucrose medium (0.1 ml.) with or without additives. The incubation was carried out aerobically with shaking at 37°. All reagents were dissolved in sucrose medium and the pH was adjusted to 7.3 before addition to the incubation flasks. Incubation was stopped after 60 min. by the addition of 3 vol. (3.6 ml.) of ice-cold 10% (w/v) trichloroacetic acid which contained a 100-fold excess of unlabelled valine.

Preparation of protein for counting. The protein precipitate was centrifuged off (at 3000g for 5 min.) and the supernatant discarded. The residue was washed three times in the centrifuge with 5.0 ml. portions of 10% (w/v) trichloroacetic acid and the washings were discarded. Nucleic acids and lipids were removed from the protein moiety by the method of Allfrey, Mirsky & Osawa (1957). The air-dried protein pellet was dissolved in 80% (v/v) formic acid (0.5 ml.) and filtered through Whatman no. 1 filter paper.

Assay of radioactivity. Samples (0.05 ml.) of protein solution in formic acid were added to flat-bottomed tubes each containing 1.1 ml. of scintillation fluid [0.4% (w/v) *p*-terphenyl and 0.04% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)-benzene in toluene-1,4-dioxan (7:3, v/v)]. The tube was arranged in a tritium coincidence-scintillation counter (Isotope Developments Ltd., model 6012) and sufficient counts were recorded to give a statistical error of less than 2%. The efficiency of the counter was 11%. All counts were corrected for self-absorption to zero thickness. Counts at zero time differed by less than 10% from background.

Protein estimation. Samples (0.05 ml.) of the protein solution in formic acid were neutralized with 5 vol. of 4.12N-NaOH and the protein content was measured by the Folin-Ciocalteu phenol method of Lowry, Rosebrough, Farr & Randall (1951), with tyrosine as standard. A factor of 4.22 was used to convert values into nuclear protein equivalent. This factor was obtained from the colour given by a trichloroacetic acid-insoluble protein mixture isolated from nuclei of rat thymus gland.

Deoxyribonucleic acid. This was estimated by the method of Burton (1956).

Firefly determination of adenosine triphosphate. Suspensions of nuclei were treated with trichloroacetic acid (final concn.: 6%, w/v) immediately after preparation to stop the degradative action of phosphatases. The protein debris was removed by centrifugation (at 3000g for 5 min.) and the acid removed by extraction with ether. All traces of ether were eliminated by aeration before the luciferin-luciferase method of Holton (1959) was used to estimate the ATP concentration. Because of inhibition by Cl^- ions present in the sucrose medium, calibration was made against standard ATP solutions prepared in the same medium.

Nuclear thiol estimation. Nuclear thiol content was assayed at pH 7.3 by the method of Ellman (1959) with GSH as standard. Nuclear suspensions (1.0 ml.) were taken and allowed to react completely (25 min. at 20°) with 5,5-dithiobis-(2-nitrobenzoic acid) (distributors: R. N. Emanuel Ltd., London). The suspensions were then centrifuged (at 3000g for 2 min.) and the extinctions of the clear supernatants measured at 412 m μ .

Thiol and disulphide estimations. These were carried out by mercurimetric amperometric titration with the dropping-mercury electrode as indicator electrode as described by Stricks, Kolthoff & Tanaka (1954). The characteristics of the capillary were: *m*, 4.0 mg.sec.⁻¹; *t*, 3.8 sec. (open circuit); *h*, 59 cm. The reference electrode was connected by a saturated KCl-agar bridge and potentials were measured relative to the saturated calomel electrode. Mercapto-ethanol and cysteamine were titrated at a potential of -0.35 v and GSH at -0.29 v. Titrations were carried out with a Radiometer PO 4 polarograph at room temperature with 10 mM-mercuric chloride solution. Determination of thiol content was carried out in a supporting electrolyte consisting of 0.05M-sodium borate buffer, pH 9.2, and 0.5M-KCl as complex-forming agent. The amount of disulphide present in the thiol solutions was given by the difference after reduction with 0.2M- Na_2SO_3 in the same electrolytic medium.

X-irradiation. This was done at the Department of Radiotherapy, Churchill Hospital, Oxford. Characteristics of the Maximar X-ray machine were: half-value layer, 1.1 mm. of Cu; operating voltage, 215 kv at 15 ma. Nuclear preparations were irradiated at 0° in plastic cups of 15 ml. capacity. The dose rate was 161 r./min. at a focal distance of 30 cm. The dose rate for irradiation *in vivo* was 28.5 r./min. at a focal distance of 64 cm. to the centre of the rat. A total dose of 1000 r. was delivered in each case.

Adrenalectomies. Rats were adrenalectomized bilaterally under Nembutal narcosis and kept in a room at 27° for 3 days after the operation. Food was available *ad libitum* and the drinking water was supplemented with NaCl (0.86%), KCl (0.04%) and glucose (2%). The animals were used after 6 days, before atrophy of the thymus commenced. No remnants of the adrenal or accessory glands were found at autopsy in any of the animals.

Radioautography. Nuclei were incubated with [³H]valine and a sample (0.1 ml.) was added after 60 min. to sucrose medium (5.0 ml.) containing a 100-fold excess of unlabelled valine and then centrifuged (at 3000g for 5 min.). The precipitate was washed twice more to ensure a low level of background activity. The nuclear pellet was finally resuspended in sucrose medium (0.2 ml.) and samples (0.05 ml.) were spread on to slides previously coated with a film of alum-gelatin (0.5 g. of chrome alum and 5 g. of gelatin/l.). Smears were air-dried for 10 min., fixed in

methanol for 15 min., rinsed in water for 10 sec. and then covered with Kodak AR 10 stripping film according to the wet technique of Fitzgerald, Simmel, Weinstein & Martin (1953). Exposures were made for 4–5 weeks in a refrigerator at 2°, at the end of which time the slides were removed, placed in Kodak D-19b developer for 5 min., fixed in Kodak acid fixer for 10 min., washed thoroughly in running tap water, rinsed in distilled water and allowed to dry. The radioautographs were then stained for 1 hr. in buffered methyl green-pyronin solution, pH 4.4, rinsed in water for 5 sec., dehydrated in acetone for 5–10 sec., immersed in acetone-xylene (1:1, v/v) for 1 min. and put into xylene for 15–20 min. All stripping-film radioautographs were examined unmounted under oil immersion in the visible-light microscope at 900–1100 $\times$ magnification. Only nuclei originating from thymocytes and which were free from visible cytoplasmic contamination were assayed for grain content and these comprised approximately 95% of the population. At least 200 labelled nuclei were assayed on each slide for grain content.

RESULTS

Nuclei prepared from rat thymus gland in sucrose medium exhibited the same requirement for glucose and sodium chloride as described (Allfrey *et al.* 1957) for calf-thymus nuclei. The optimum concentration of sodium chloride in this case was 20–30 mM and stimulated amino acid incorporation threefold. The addition of an amino acid complement, comprising 14 different L-amino acids (excluding valine) each in 10 μM concentration, was relatively inactive in stimulating the uptake of [³H]valine into nuclear proteins; a maximum increase of 20% was obtained. The intranuclear amino acid pool is probably of sufficient size to allow protein synthesis to proceed for the duration of the incubation (60 min.).

Herranen & Brunkhorst (1962) noticed a direct relationship between the protein-synthetic ability of nuclei prepared from rabbit thymus glands that had been removed from the animal at various times after death. We confirmed this finding in our studies with the rat thymus and measured the simultaneous disappearance of intranuclear ATP (Osawa, Allfrey & Mirsky, 1957), which may explain the observed decrease in amino acid incorporation (Table 1). Because of these rapid post-mortem changes it is necessary to control the time of removal of the gland carefully if comparisons between different groups of animals are to be made. In our studies the glands were removed exactly 1 min. after cervical fracture, to produce nuclei of high synthetic activity, or after 10 min. when the rate of change is less rapid. Apart from the absolute level in activity the two times gave results that were essentially the same. At least four and usually six animals were used in each experiment to minimize the effects of individual variation.

Attempts were made to 'revive' the aged nuclei by the addition of ATP. Concentrations in the range 0.1–5.0 mM produced no significant stimulation. Inhibition of amino acid incorporation occurred at the higher ATP concentrations (Table 2), which may be explained by the loss of nuclear structure observable in the light microscope after staining with methyl green–pyronin. An assay of thiol content in nuclei after treatment with ATP showed that a decrease of reacting thiol groups occurred (Table 2). This apparent relationship between thiol content and nuclear protein synthesis was therefore investigated. All reagents that react with or tend to oxidize thiol groups to disulphide groups inhibited amino acid incorporation

(Table 3). Of these, the specific thiol inhibitors chloromercuribenzenes-*p*-sulphonate and *N*-ethylmaleimide were the most potent and caused a complete inhibition of protein synthesis. The alkylating agent iodoacetamide and the mercaptide-forming inhibitor Mapharsan (2-aminoarsenosphenol hydrochloride) both diminished protein synthesis successfully, but the inhibition caused by the oxidizing agents, e.g. porphyrindin, dichlorophenol-indophenol and GSSG, was variable. These results implicate thiol as a functional group in the reactions leading to protein synthesis.

Reagents with a reducing capacity such as GSH and cysteamine, on the other hand, had a slight stimulatory effect on protein synthesis, and when GSSG and GSH were added together the individual effects previously produced by these reagents cancelled each other out. The slight decrease in activity noticed when the reducing agent mercaptoethanol was added to the nuclei was unexpected (cf. Schram & Brachet, 1962). It is known that thiol compounds can be oxidized on standing in air and an amperometric estimation of disulphide was carried out to determine if any of the thiol solutions used were contaminated by disulphides. All the thiol solutions contained some disulphides (Table 4), but the degree of contamination was highest (8.65%) in the mercaptoethanol solution and this may explain the observed effect.

Nuclear preparations that had been irradiated with 1000 r. X-rays showed a decreased ability to incorporate amino acids into proteins (Fig. 1). These effects were first noticed by other workers (Logan,

Table 1. *Relationship between time of removal of the thymus gland from the animal, the adenosine triphosphate content of nuclei and the synthetic activity*

Thymus glands were removed from the animals at intervals after cervical fracture. Nuclei were prepared in sucrose medium [sucrose (0.25 M) and CaCl_2 (3.3 mM) in 5 mM-tris-HCl, pH 7.3] and incubated with DL-[^3H]valine for 1 hr. in the presence of glucose and NaCl. Incorporated radioactivity was assayed in a coincidence-scintillation counter. ATP estimations were made by the luciferin-luciferase method after the proteins had been precipitated with trichloroacetic acid. Experimental details are described in the text. The results are given as means $\pm$ S.D., with the numbers of experiments in parentheses.

Time from decapitation to removal of the thymus (min.)	ATP content ($\mu\text{m-moles/mg. of DNA P}$)	Incorporated radioactivity (counts/min./ $\mu\text{g. of protein}$)
0.5	137 $\pm$ 23 (2)	31.7 $\pm$ 2.1 (2)
1.0	71 $\pm$ 18 (7)	21.5 $\pm$ 2.3 (4)
2.0	59 $\pm$ 2 (2)	17.5 $\pm$ 0.2 (2)
5.0	35 $\pm$ 2 (2)	13.8 $\pm$ 0.1 (2)
10.0	23 $\pm$ 5 (7)	11.4 $\pm$ 3.1 (4)

Table 2. *Effects of added adenosine triphosphate on protein synthesis and thiol content in the nucleus*

Nuclei were prepared in sucrose medium at pH 7.3 (see Table 1). The effects of ATP in different concentrations on the incorporation of [^3H]valine into nuclear proteins was assayed in a scintillation counter. The pH of ATP solutions was adjusted to 7.3 before addition to the incubation flasks. Thiol content was measured by the method of Ellman (1959). The results are given as means $\pm$ S.D. of two independent determinations.

Concn. of ATP (mM)	Incorporated radioactivity (% of control)	Nuclear thiol content ($\mu\text{m-moles/mg. of DNA P}$)
0.0	100	446 $\pm$ 4
0.1	99 $\pm$ 4	439 $\pm$ 1
0.5	97 $\pm$ 2	422 $\pm$ 3
1.0	88 $\pm$ 5	410 $\pm$ 6
5.0	71 $\pm$ 5	372 $\pm$ 9

Table 3. *Influence of thiol reagents on amino acid incorporation in nuclei from rat thymus gland*

Nuclear suspensions (1.0 ml.) were incubated in sucrose medium (see Table 1) containing glucose (20 mM), NaCl (20 mM) and [^3H]valine (10 $\mu\text{C}/\text{ml.}$) together with added reagents in a final concentration of 1 mM and volume of 1.2 ml. The pH of the incubation medium after addition of the reagents was 7.3. Radioactivity incorporated into acid-insoluble nuclear proteins was assayed after incubation for 60 min. at 37°. The results are given as means $\pm$ S.D., with the numbers of estimations in parentheses.

Reagent added	Incorporated activity (% of control)
None	100.0
Chloromercuribenzenes- <i>p</i> -sulphonate	0.6 $\pm$ 0.6 (3)
<i>N</i> -Ethylmaleimide	0.4 $\pm$ 0.2 (2)
Iodoacetamide	8.1 $\pm$ 0.4 (3)
Mapharsan	7.5 $\pm$ 1.1 (2)
Porphyrindin	30.0 $\pm$ 6.1 (2)
Dichlorophenol-indophenol	2.1 $\pm$ 1.2 (2)
GSSG	91.0 $\pm$ 3.0 (4)
GSH	111.0 $\pm$ 6.0 (4)
GSSG + GSH	100.5 $\pm$ 1.5 (2)
Cysteamine	113.0 $\pm$ 8.0 (4)
Mercaptoethanol	95.0 $\pm$ 2.0 (3)

Errera & Ficq, 1959; Faurès & Errera, 1962) in their radioautographic studies. The similarity of our observations indicates that comparable results are obtained by grain counting of radioautographic films and by direct assay of the incorporated radioactivity in a counter.

Since it was observed (Table 5) that there was a diurnal variation in the synthetic ability of nuclei, nuclear preparations for control and experimental observations were made immediately after each other in the morning when the rate of change is less variable. Groups of animals were either irradiated at intervals

and killed as soon as possible after each other, or control groups were included at each time-interval. Animals were not sham-irradiated but were placed behind a lead shield in the same room during the irradiation of the other rats. In the irradiation experiments two sets of results were obtained, one from nuclei prepared from glands removed 1 min. after decapitation of the rats and another from glands processed 10 min. after death. Apart from slight differences the two sets of results were essentially the same (Table 6). Whole-body X-irradiation of normal rats produced an immediate stimulation in protein synthesis (Table 6). The enhanced synthesis, however, was transient and a significant decrease in synthesis manifested itself 2 hr. after irradiation. To eliminate some of the hormonal changes produced by stresses caused by, among other things, X-irradiation, the above experiment was repeated with rats from which the adrenal glands were removed 6 days earlier. In this case a decrease in protein synthesis occurred 30 min. after irradiation, increasing to 44–64 % after 2 hr. (Table 6). Some of these results were confirmed by radioautographic studies (Table 7), which had the advantage that nuclei with attached cytoplasmic debris and whole cells could be excluded from the determination. Only nuclei originating from the thymocyte cell and which appeared morphologically pure after staining with methyl green-pyronin under the light microscope were assayed for grain content and comprised about 95 % of the total population.

Table 4. *Disulphide contamination of thiol solutions used*

A mercurimetric amperometric titration of samples (1.0 ml.) of approx. 10 mM-thiol solutions was carried out with 10 mM-mercuric chloride according to the method of Stricks, Kolthoff & Tanaka (1954). The supporting electrolyte was 0.05 M-borax buffer, pH 9.2, and 0.5 M-KCl. Disulphides were reduced with 0.2 M- Na_2SO_3 and the difference in thiol content before and after reduction measured the disulphide contamination. Each value is the average of two determinations.

	Thiol content (mM)	Thiol content after reduction (mM)	Disulphide present (%)
Mercaptoethanol	8.815	9.650	8.65
Cysteamine	9.164	9.423	2.75
GSH	9.006	9.452	4.72

Fig. 1. Protein synthesis in irradiated nuclei. Nuclear suspensions in sucrose medium (see Table 1) were irradiated at 0° with different doses of X-rays and then incubated with [^3H]valine in the presence of glucose and NaCl. Trichloroacetic acid-insoluble proteins were precipitated with trichloroacetic acid and the incorporated radioactivity was assayed after unused [^3H]valine had been washed off.

DISCUSSION

The ATP requirement in nuclear protein synthesis was shown conclusively by Allfrey & Mirsky (1961): all reagents that blocked ATP synthesis in the nucleus also blocked the incorporation of amino acids into proteins. Hoagland (1955)

Table 5. *Relationship between the ability of nuclei to synthesize protein and the time of day*

Four rats were used/group and nuclei were prepared from them at intervals during the day. Thymus glands were removed at 1 min. after decapitation. Incorporation of [^3H]valine was allowed to proceed for 60 min. at 37° and the reaction was terminated by the addition of ice-cold trichloroacetic acid. The trichloroacetic acid-insoluble proteins were washed free from unused [^3H]valine and the incorporated radioactivity was assayed. The results are given as means $\pm$ S.D., with the numbers of experiments in parentheses.

Time of day	Incorporated radioactivity (counts/min./ μg . of protein)
9.30	20.8 $\pm$ 2 (2)
11.30	17.3
14.30	45.6 $\pm$ 5 (2)
17.30	27.6 $\pm$ 4 (2)

demonstrated that the 'high-energy' phosphate is required in the activation of amino acids in the cytoplasm and a similar requirement exists for the activation reaction in the rat-thymus nucleus (Smit & Stocken, 1963*b*). The difference between the ribosomal and the nuclear systems seems to be that protein synthesis in the latter is not stimulated by added ATP. Similar observations have been made by previous workers (Allfrey & Mirsky, 1961; Rees & Rowland, 1961) in their studies with calf-thymus and rat-liver nuclei. Many models to explain the action of ATP can be constructed. The

formation of complexes with Ca^{2+} and Mg^{2+} ions (Burton, 1959) removes ions that are considered important in the maintenance of chromosome (Mazia, 1954) and nuclear (Allfrey *et al.* 1957) structure. We noticed a loss of nuclear structure after treatment with ATP. The decrease in thiol content observed may be caused by the oxidation of thiol groups to disulphide groups stimulated in the presence of ATP or by a spatial rearrangement of released proteins. Until more is known about these intranuclear reactions, speculation about the mechanism does not appear to be very useful.

Table 6. *Effects of irradiation in vivo on protein synthesis in nuclei prepared from normal and adrenalectomized rats*

Groups of four to six rats were used for each experimental value and received 1000 r. X-rays at intervals. Animals were killed at $\frac{1}{2}$ hr. intervals after irradiation and the thymus glands removed after 60 ± 1 sec. or 10 min. for the preparation of nuclei. Nuclear suspensions in sucrose medium (see Table 1) were incubated with [^3H]valine in the presence of glucose (20 mM) and NaCl (20 mM) and the incorporated radioactivity was assayed after incubation for 60 min.

	Time after irradiation (hr.)	Incorporated radioactivity (% of control)	
		Glands removed after 1 min.	Glands removed after 10 min.
Normal rats	0.5	107, 116, 124	103, 111
	1.0	101, 102, 105, 112, 117	102, 105
	1.5	92, 96	74, 82
	2.0	38, 49	55, 56
Adrenalectomized rats	0.5	89, 89	75, 75
	1.0	71, 77	68
	1.5	53, 61	67
	2.0	30, 41	56
Incorporated radioactivity (counts/min./ $\mu\text{g.}$ of protein)			
Normal non-irradiated rats		21.5, 22.3, 20.8, 22.8, 23.0, 21.9	12.9, 12.8, 11.7, 13.3, 12.9, 13.7
Adrenalectomized non-irradiated rats		26.2, 23.3, 23.6	13.6, 14.1, 14.8

Table 7. *Effects of whole-body X-irradiation on protein synthesis in nuclei isolated from normal and adrenalectomized rats as studied by radioautography*

Rats were irradiated with 1000 r. X-rays, the animals killed at intervals and nuclei prepared from glands removed after 60 sec. Incubation was carried out in sucrose medium (see Table 1) in the presence of [^3H]valine for 1 hr. Incorporated radioactivity was assayed by radioautography. Grain counts for each value were made on at least 200 nuclei. The grains present in an area of film of equal size and immediately next to labelled nuclei were subtracted as background. This background value was always less than 1 grain/nucleus.

Time after irradiation (hr.)	Dose (r.)	Grains/nucleus (% of total)					Average grains/ nucleus
		0	1-2	3-5	6-10	> 10	
Normal rats							
—	0	54	34	6	4	2	1.34
0.5	1000	43	39	10	5	3	1.80
1.0	1000	55	32	8	3	2	1.33
2.0	1000	60	29	7	3	1	1.08
Adrenalectomized rats							
—	0	45	32	12	6	6	2.07
0.5	1000	53	30	10	4	3	1.55
1.0	1000	54	34	7	3	2	1.18
2.0	1000	60	30	6	2	2	1.09

Reports on the role played by thiol enzymes in reactions leading to protein synthesis are numerous. The purified alanine-activating (Holley & Goldstein, 1959) and tryptophan-activating (Davie, Koningsberger & Lipmann, 1956) enzymes isolated from liver or pancreas are inhibited by *p*-chloromercuribenzoate and air-oxidation. A thiol function is also evident from the results reported by Hülsmann & Lipmann (1960), Bishop & Schweet (1961) and Rendi & Hultin (1960) on the transfer reaction of amino acids from transfer RNA to microsomal protein. The activity of their microsomal preparations was inhibited by *p*-chloromercuribenzoate, *N*-ethylmaleimide and reagents that oxidized thiol groups, and a stimulation was given by GSH and cysteamine. These observations and our results on nuclear protein synthesis emphasize the role played by thiol groups in reactions governing protein synthesis.

Edelmann (1951) first noticed that adrenalectomized rats are more sensitive to irradiation than normal animals. It is known that whole-body irradiation inhibits the release of prolactin, gonadotrophic hormone and growth hormone from the anterior pituitary gland but enhances the secretion of adrenocorticotrophic hormone which acts on the adrenal gland. Since 50 % of the stored adrenaline can be secreted into the bloodstream after subjection to stress there is an immediate effect on metabolic rates. These reports may explain our finding that there is a stimulation in nuclear protein synthesis in normal rats immediately after irradiation but not when adrenalectomized animals are used.

Various explanations can be offered to explain the radiation damage noticed in our studies on nuclear protein synthesis. Any process that decreases the intranuclear ATP content such as the increased adenosine-triphosphatase activity noticed by Dubois & Petersen (1954) in rat thymus gland after irradiation will impair protein synthesis (Allfrey *et al.* 1957). The synthesis of ATP and nuclear phosphorylation in thymus nuclei are abolished by X-ray doses as low as 100 r. (Creasey & Stocken, 1959; Klouwen & Betel, 1963). A decrease in nuclear amino acid activation on irradiation (Smit & Stocken, 1963*b*) may account for the diminished incorporation of amino acids into protein. Evidence provided by Faurès & Errera (1962) shows that some fundamental damage is caused in the DNA molecule by 300 r. X-rays, and that the inhibition in nuclear protein synthesis that results can be restored by the addition of denatured DNA. They implicated cations such as Na⁺ and K⁺ ions as stabilizers for the nucleic acid structure. These ions are indispensable for amino acid uptake into nuclei (Allfrey *et al.* 1957) but are released from the thymus nucleus with small doses of X-rays

(Creasey, 1960). An investigation of the radio-mimetic action of GSSG on nuclei isolated from rat thymus gland has shown that 0.1 mM inhibits glucose 6-phosphate dehydrogenase (E.C. 1.1.1.49), the uptake of [³²P]phosphate into RNA and the formation of labile phosphate (Ord & Stocken, 1963). The irradiation of nuclei *in vitro* decreases their thiol content, but if nuclei are isolated from rats given 1000 r. whole-body X-irradiation there seems to be a concomitant increase in fixed thiol groups which are capable of reacting with 1-¹⁴C-labelled *N*-ethylmaleimide (Deakin, Ord & Stocken, 1963). These results suggest that the most likely primary cause of the biochemical derangement is the alteration produced in the thiol-disulphide equilibrium and it may well be the cause of the decreased amino acid incorporation into nuclear proteins observed by us.

SUMMARY

1. Protein synthesis in nuclei was inhibited by the addition of ATP. Damage to the nuclear structure occurred and the intranuclear thiol content was decreased.

2. Enhanced protein synthesis was caused by reducing agents. Reagents that oxidized or reacted with thiol groups were inhibitory.

3. Irradiation of isolated nuclei inhibited incorporation of amino acids into proteins.

4. Nuclear protein synthesis was stimulated immediately after whole-body X-irradiation. Nuclei prepared from adrenalectomized rats reacted differently on irradiation and showed a decreased synthesis. Protein synthesis was inhibited in both instances 2 hr. after irradiation.

We thank the Department of Scientific and Industrial Research, the Rockefeller Foundation and the National Institutes of Health (Grant no. A-3369) for grants in aid of equipment expenses, and also the South African Atomic Energy Board who supported J.A.S.

REFERENCES

- Abrams, R. (1951). *Arch. Biochem.* **30**, 90.
- Allfrey, V. G. & Mirsky, A. E. (1961). In *Protein Biosynthesis*, p. 49. Ed. by Harris, R. J. C. London: Academic Press (Inc.) Ltd.
- Allfrey, V. G., Mirsky, A. E. & Osawa, S. (1957). *J. gen. Physiol.* **40**, 451.
- Bacq, Z. M. & Alexander, P. (1961). *Fundamentals of Radiobiology*, p. 387. Oxford: Pergamon Press Ltd.
- Bekkum, D. W. & Cohen, J. A. (1955). *Proc. 1st U.N. int. Conf. on the Peaceful Uses of Atomic Energy, Geneva*, vol. 11, p. 383.
- Bishop, J. O. & Schweet, R. S. (1961). *Biochim. biophys. Acta*, **49**, 235.
- Bransome, E. D. & Reddy, W. J. (1963). *Arch. Biochem. Biophys.* **101**, 21.

- Burton, K. (1956). *Biochem. J.* **62**, 315.
- Burton, K. (1959). *Biochem. J.* **71**, 388.
- Butler, J. A. V., Cohn, P. & Crathorn, A. R. (1957). In *Advances in Radiobiology*, p. 33. Edinburgh: Oliver and Boyd.
- Creasey, W. A. (1960). *Biochim. biophys. Acta*, **38**, 181.
- Creasey, W. A. & Stocken, L. A. (1959). *Biochem. J.* **72**, 519.
- Davie, E. W., Koningsberger, V. V. & Lipmann, F. (1956). *Arch. Biochem. Biophys.* **65**, 21.
- Deakin, H., Ord, M. G. & Stocken, L. A. (1963). *Biochem. J.* **89**, 296.
- Dubois, K. P. & Petersen, D. F. (1954). *Amer. J. Physiol.* **176**, 282.
- Edelmann, A. (1951). *Amer. J. Physiol.* **167**, 345.
- Ellman, G. L. (1959). *Arch. Biochem. Biophys.* **82**, 70.
- Faurès, E. & Errera, M. (1962). *Int. J. Radiat. Biol.* **4**, 477.
- Fitzgerald, P. J., Simmel, E., Weinstein, J. & Martin, C. (1953). *Lab. Invest.* **2**, 181.
- Hempelmann, L. H., Carr, S., Frantz, I. D., Masters, R. & Lamdin, E. (1950). *Fed. Proc.* **9**, 183.
- Herranen, A. & Brunkhorst, W. (1962). *Biochim. biophys. Acta*, **65**, 523.
- Hevesy, G. (1949). *Nature, Lond.*, **163**, 869.
- Hoagland, M. B. (1955). *Biochim. biophys. Acta*, **16**, 288.
- Holley, R. W. & Goldstein, J. (1959). *J. biol. Chem.* **234**, 1765.
- Holmes, B. E. & Mee, L. K. (1952). *Brit. J. Radiol. N.S.* **25**, 273.
- Holton, P. (1959). *J. Physiol.* **145**, 494.
- Hüllsmann, W. C. & Lipmann, F. (1960). *Biochim. biophys. Acta*, **43**, 123.
- Kay, R. E. & Entenman, C. (1956). *Arch. Biochem. Biophys.* **62**, 419.
- Killander, D., Ribbing, C., Ringertz, N. R. & Richards, B. M. (1962). *Exp. Cell Res.* **27**, 63.
- Klouwen, H. M. & Betel, I. (1963). *Int. J. Radiat. Biol.* **6**, 441.
- Logan, R., Errera, M. & Ficq, A. (1959). *Biochim. biophys. Acta*, **32**, 147.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Mazia, D. (1954). *Proc. nat. Acad. Sci., Wash.*, **40**, 521.
- Ord, M. G. & Stocken, L. A. (1963). *Nature, Lond.*, **200**, 136.
- Osawa, S., Allfrey, V. G. & Mirsky, A. E. (1957). *J. gen. Physiol.* **40**, 491.
- Rees, K. R. & Rowland, G. F. (1961). *Biochem. J.* **78**, 89.
- Rendi, R. & Hultin, T. (1960). *Exp. Cell Res.* **19**, 253.
- Richmond, J. E., Altman, K. I. & Salomon, K. (1951). *J. biol. Chem.* **190**, 817.
- Richmond, J. E., Ord, M. G. & Stocken, L. A. (1957). *Biochem. J.* **66**, 123.
- Salganik, R. I. (1958). *Biokhimiya*, **23**, 377.
- Schram, A. & Brachet, J. (1962). *Biochim. biophys. Acta*, **57**, 596.
- Selye, H. (1957). *The Stress of Life*, p. 227. London: Longmans, Green and Co.
- Smit, J. A. & Stocken, L. A. (1963a). *Biochem. J.* **89**, 10p.
- Smit, J. A. & Stocken, L. A. (1963b). *Biochem. J.* **89**, 37.
- Stricks, W., Kolthoff, I. M. & Tanaka, N. (1954). *Analyt. Chem.* **26**, 299.

Biochem. J. (1964) **91**, 161

Apparatus for Rapid and Sensitive Spectrophotometry

By Q. H. GIBSON* AND L. MILNES

Department of Biochemistry, University of Sheffield, Sheffield 10

(Received 29 May 1963)

Although the first application of flow methods for following a rapid chemical reaction in solution was made by Hartridge & Roughton (1923) during work on a biochemical problem, and although many of the technical advances in the meantime have been dictated by special biochemical requirements (for reviews see Roughton, 1953; Chance, 1953), the methods have been restricted to a very few Laboratories. The apparatus described in the present paper is the result of several years' experience and is intended for work with clear solutions with extinction changes of 0.02–0.5 in observation tubes of 2–20 mm. optical path. The aim has been to build a robust and reliable apparatus for use in general

biochemical work. The need for reliability is particularly evident with enzymes where a week's work may be needed to yield enough material for a single set of kinetic determinations. The apparatus described does not exist only as a single example; ten complete sets are already in use in different Laboratories and it is hoped that the description is full enough to be followed by any competent mechanic.

CONSTRUCTION

Layout and principle of operation. This may be followed by reference to Figs. 1 and 2. The reagents are placed in the 25 ml. reservoir syringes (*A* in Figs. 1 and 2) from which they are transferred by proper setting of the valves to the 2 ml. driving syringes (*B* in Figs. 1 and 2). To make a determination the driving syringes are forced in together

* Present address: Johnson Research Foundation, University of Pennsylvania, Philadelphia 4, Pa., U.S.A.