3. In some species considerable differences can be recognized between different tissues, although underlying species characteristics can always be recognized.

4. The patterns persist in cells which have been cultured *in vitro* for many years and are therefore genetically stable.

5. It is concluded that an enormous number of proteins with the same functional activity can exist and the significance of this observation is discussed.

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Anti-Inflammatory Drugs and Intermediary Metabolism

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Salicylate uncouples oxidative-phosphorylation reactions in respiring mitochondrial preparations (Brody, 1956), and many of its effects on the meta-

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bolism of animals and isolated tissues are explicable in terms of this action (Smith, 1959; Smith & Moses, 1960). However, the anti-inflammatory properties of salicylate must be mediated by mechanisms other than an uncoupling action, since 2:4-dinitrophenol, although a more powerful uncoupling reagent than salicylate, lacks anti-

inflammatory effects (Adams & Cobb, 1958; Marks & Smith, 1960). The present study is concerned with the effects of four other anti-inflammatory drugs, butazolidin (3:5-dioxo-1:2-diphenyl-4-*n*-butylpyrazolidine), chloroquine diphosphate [7-chloro-4-(4-diethylamino-1-methylbutylamino)-quinoline diphosphate], cortisol (11 β :17 α :21-trihydroxypregn-4-ene-3:20-dione) and dexamethasone (16 α -methyl-9 α -fluoro-11 β :17 α :21-trihydroxypregna-1:4-diene-3:20-dione) on the incorporation of ^{14}C from [^{14}C]glucose, [$3\text{-}^{14}\text{C}$]pyruvate and [$2\text{-}^{14}\text{C}$]acetate into the soluble metabolic intermediates of chopped preparations of rat liver and brain. The work was undertaken to determine if these substances, together with salicylate, share any common actions on tissue metabolism which may possibly be related to their effects against inflammatory processes. A preliminary account of this work has been published (Moses & Smith, 1960).

EXPERIMENTAL

The techniques used were those described by Smith & Moses (1960) with the following modifications, which were designed to reduce the amounts of radioactive substrate and X-ray film used in each experiment and also to increase the ratio of labelled substrate to tissue in the incubation mixtures. Portions (0.25 ml.) of the final chopped-tissue suspension (containing either 7.2 mg. dry wt. of liver or 0.95 mg. dry wt. of brain) were dispensed into tubes (8.0 cm. length; 1.4 cm. diam.). Each tube also contained 50 μl . of the incubation medium described by Smith & Moses (1960) in the control experiments, or 50 μl . of a solution of one of the drugs in this medium. The concentrations of the drugs, after admixture with the tissue suspensions, were 10 μg ./ml. for butazolidin, chloroquine diphosphate and cortisol, and 0.5 μg ./ml. for dexamethasone. A solution of the radioactive substrate (5 μl .) was then added as [^{14}C]glucose (5 μC , 0.078 μmole), [$3\text{-}^{14}\text{C}$]pyruvate (5 μC , 1.38 μmole) or [$2\text{-}^{14}\text{C}$]acetate (5 μC , 1.01 μmole); labelled pyruvate was not used with the brain

preparations. The radioactive materials were obtained from The Radiochemical Centre, Amersham, Bucks. After incubation of the tubes for 3 hr. at 37° in an atmosphere of air, 1 ml. of absolute ethanol was added to each tube and the mixture was heated to boiling, cooled and centrifuged for 5 min. at about 1600 g. The supernatant was retained and the residue extracted twice by the addition of portions (0.3 ml.) of boiling 20% (v/v) ethanol, followed by centrifuging. The combined supernatants were made up to a volume of 2 ml. with absolute ethanol and portions (0.4 ml.) were analysed for the radioactive substances present by the techniques described previously (Smith & Moses, 1960). Separate analyses of the cell extract and incubation medium were not performed since the earlier work had shown that there was little, if any, difference between their patterns of radioactive intermediates. Phosphate esters were not analysed separately as they did not contain sufficient radioactivity to justify a detailed analysis.

RESULTS

Total incorporation of ^{14}C from glucose, pyruvate and acetate into soluble intermediates of liver and brain. The results given in Table 1 show that although there was no consistent effect of the drugs on the total incorporation of ^{14}C into the soluble metabolites by liver from [^{14}C]glucose, all the substances stimulated the total uptake of the isotope from the labelled glucose by the brain, dexamethasone being the most active. There were no appreciable effects of the drugs on the total incorporation of radiocarbon from [$3\text{-}^{14}\text{C}$]pyruvate by liver, but the corresponding uptakes from the labelled acetate were inhibited in both tissues, dexamethasone again being the most active substance.

Distribution of ^{14}C in the soluble intermediates. The results in Tables 2 and 3 show the amounts of ^{14}C incorporated into the soluble intermediates of the liver from labelled pyruvate and acetate, and into those of the brain from the labelled glucose and acetate, in the presence and absence of the four drugs.

Table 1. *Effects of butazolidin, chloroquine diphosphate, cortisol and dexamethasone on the total incorporation of ^{14}C from [^{14}C]glucose, [$3\text{-}^{14}\text{C}$]pyruvate and [$2\text{-}^{14}\text{C}$]acetate into the soluble intermediates of rat liver and brain*

Each tube contained 0.25 ml. of tissue suspension (7.2 mg. dry wt. of liver or 0.95 mg. dry wt. of brain) in the presence or absence of 10 μg ./ml. of butazolidin, chloroquine diphosphate or cortisol, or of 0.5 μg ./ml. of dexamethasone. The tissue was incubated with 5 μC of labelled substrate for 3 hr. at 37°. One-fifth of the total volume of extract was used for chromatography on each sheet of paper and the total counts/min. in each extract were obtained from the sum of the counts of all the radioactive spots on the paper excluding that due to residual substrate. Results are expressed as percentages of the ^{14}C incorporated into the sum of all the soluble intermediates compared with the values observed in the absence of the drugs.

Drug	Liver			Brain	
	[^{14}C]Glucose	[$3\text{-}^{14}\text{C}$]Pyruvate	[$2\text{-}^{14}\text{C}$]Acetate	[^{14}C]Glucose	[$2\text{-}^{14}\text{C}$]Acetate
Butazolidin	108	95	89	119	106
Chloroquine diphosphate	117	114	72	120	84
Cortisol	98	100	87	121	82
Dexamethasone	94	94	60	137	77
10 $^{-3}$ $\times$ Counts/min./25 mg. dry wt. of tissue in absence of drugs	150	830	290	550	400

Table 2. *Metabolism of [3-¹⁴C]pyruvate and [2-¹⁴C]acetate by rat liver in the presence and absence of four anti-inflammatory drugs*

Each tube contained 7.2 mg. dry wt. of liver in a total volume of 0.305 ml. The anti-inflammatory drugs were present in the following concentrations: butazolidin, chloroquine diphosphate and cortisol, 10 μ g./ml.; dexamethasone, 0.5 μ g./ml. The tissue was incubated with 5 μ C of labelled substrate for 3 hr. at 37°. The Table shows the ¹⁴C present in the whole sample, expressed as 10⁻³ × counts/min./25 mg. dry wt. of tissue.

Labelled substrate ...	[3- ¹⁴ C]Pyruvate					[2- ¹⁴ C]Acetate				
	None	Buta- zolidin	Chloroquine	Cortisol	Dexa- methasone	None	Buta- zolidin	Chloroquine	Cortisol	Dexa- methasone
Anti-inflammatory drug ...										
Sugar phosphates and uridine diphosphate glucose	3.1	2.6	2.0	2.4	3.2	4.8	4.6	2.6	2.8	2.0
Phosphoglyceric acid	0.9	0.9	0.9	0.9	0.5	0.5	0.6	0.1	0.4	0.4
Phosphoenolpyruvic acid	1.2	1.6	1.8	1.1	2.1	0.3	0.4	0.1	0.2	0.2
Nucleotides	1.6	1.1	0.2	0.5	0.6	1.9	1.8	1.7	1.4	1.1
Maltose	0	0	0	0	0	1.5	1.0	0.8	1.1	0
Aspartic acid	20	12	16	21	15	33	26	20	29	17
Glutamic acid	230	160	210	220	170	84	83	67	76	56
Alanine	310	270	340	270	230	8.4	9.3	6.5	10	6.8
γ -Aminobutyric acid	11	8.4	5.8	11	17	18	16	11	14	7.9
Glutamine	33	32	17	31	32	30	31	22	30	22
Lactic acid	130	190	190	180	230	70	54	51	56	42
Succinic acid	8.7	19	21	8.1	12	3.4	2.3	2.0	2.8	2.2
Malic acid	0	0	0.2	0	0	13	11	8.3	13	8.4
Citric acid	1.0	2.0	1.2	1.0	1.4	0.5	0.5	0.3	0.6	0.2
Glyceric acid	28	17	32	16	16	0	0	0	0	0
Total in:										
Sugar phosphates	5.2	5.1	4.7	4.4	5.8	5.6	5.6	2.8	3.4	2.5
Nucleotides	1.6	1.1	0.2	0.5	0.6	1.9	1.8	1.7	1.4	1.1
Maltose	0	0	0	0	0	1.5	1.0	0.8	1.1	0
Amino acids	610	500	620	580	480	190	180	140	170	120
Organic acids	190	260	280	220	260	87	68	62	72	53
Other substances	30	32	41	33	32	4.0	2.0	0.6	2.3	1.5
Soluble extract	830	800	950	830	780	290	260	210	250	170

Table 3. *Metabolism of [¹⁴C]glucose and [^{2-¹⁴C]acetate by rat brain in the presence and absence of four anti-inflammatory drugs}*
 Details were as given for Table 2, except that each tube contained 0.95 mg. dry wt. of brain.

Labelled substrate	Anti-inflammatory drug	[¹⁴ C]Glucose					[2- ¹⁴ C]Acetate				
		None	Buta- zolidin	Chloroquine	Cortisol	Dexa- methasone	None	Buta- zolidin	Chloroquine	Cortisol	Dexa- methasone
Sugar phosphates, uridine glucose and nucleotides	diphosphate	150	180	250	240	240	0	0	0	0	0
Aspartic acid		1200	1300	1300	1300	1400	140	160	110	100	98
Glutamic acid		980	1100	1100	1200	1300	110	120	110	120	110
Alanine		900	880	950	1100	1300	9.5	9.5	4.1	0	0
γ-Aminobutyric acid		440	590	600	500	730	40	48	33	32	35
Glutamine		580	700	680	710	780	0	0	0	0	0
Lactic acid		34	96	83	46	21	3.9	0	0	0	0
Succinic acid		330	400	300	240	82	4.6	3.8	6.6	6.3	6.3
Malic acid		140	200	200	200	240	36	30	12	21	21
Citric acid		490	520	410	590	610	34	35	28	22	25
Total in											
Phosphate esters		150	180	250	240	240	0	0	0	0	0
Amino acids		4300	4700	4800	5100	5900	300	340	260	260	240
Organic acids		990	1200	990	1100	960	78	69	46	54	53
Other substances		110	450	510	350	470	22	22	35	19	14
Soluble extract		5500	6500	6500	6700	7500	400	430	340	330	310

None of the drugs affected the percentage distribution of radioactivity in the principal components of the soluble intermediates derived from these labelled substrates in the two tissues. However, all four anti-inflammatory substances showed marked effects on the distribution of radiocarbon among the soluble intermediates of liver incubated with [¹⁴C]glucose (Table 4). The incorporation of the isotope into alanine, which was the most prominently labelled substance in the corresponding control experiment, was greatly decreased in the presence of the drugs, whereas increased amounts of radioactivity were found in maltose, glyceric acid, phosphoglyceric acid and lactic acid. In addition, ¹⁴C was detected in glutamic acid and nucleotides in the presence of the drugs, whereas it was not observed in these compounds in the control sample. In this tissue approximately 94% of the labelled glucose was recovered unchanged at the end of the experiment in the presence or absence of the drugs.

DISCUSSION

The results show that butazolidin, chloroquine diphosphate, cortisol and dexamethasone produced very similar effects on the incorporation of ¹⁴C from labelled substrates into the soluble intermediates of rat liver and brain. These four drugs, of diverse chemical structure, therefore share both anti-inflammatory properties and common actions on intermediary metabolism. However, when the present results are compared with those obtained with salicylate in similar experiments (Smith & Moses, 1960), it is apparent that there are few points of similarity. All the drugs used in the present work, as well as salicylate, caused an increased total incorporation of ¹⁴C from labelled glucose into the soluble intermediates of brain, and decreased the uptake of radioactivity from [2-¹⁴C]-acetate in both liver and brain. The inhibitory effects of salicylate in the latter instance were much more pronounced than with the other four drugs. The most striking differences were concerned with the distribution of ¹⁴C among the various metabolic intermediates. Thus salicylate produced little, if any, effect on the distribution of the isotope in liver incubated with [¹⁴C]glucose, whereas the other drugs caused a considerable decrease in the amount of radiocarbon in alanine, and this was accompanied by a relative accumulation of ¹⁴C in maltose, phosphoglycerate and glycerate. In liver incubated with [3-¹⁴C]pyruvate, salicylate increased the incorporation of radioactivity into glutamic acid and decreased its entry into alanine (A. K. Huggins, M. J. H. Smith & V. Moses, unpublished work); none of the other drugs had any effect in this system. It must therefore be concluded that the action of salicylate on tissue

Table 4. *Metabolism of [^{14}C]glucose by rat liver in the presence and absence of four anti-inflammatory drugs*

Details were as given for Table 2.

Anti-inflammatory drug ...	None	Butazolidin	Chloroquine	Cortisol	Dexamethasone
Sugar phosphates and uridine diphosphate glucose	13	15	14	11	13
Phosphoglyceric acid	0.8	4.0	3.9	3.6	2.3
Phosphoenolpyruvic acid	0	0	0	0	0
Nucleotides	0	11	13	12	11
Maltose	9.8	57	66	53	52
Aspartic acid	8.6	13	14	8.9	12
Glutamic acid	0	1.3	2.2	2.2	2.2
Alanine	98	18	19	16	11
γ -Aminobutyric acid	7.3	7.2	9.7	7.0	7.0
Glutamine	0	0	0	0	0.9
Lactic acid	0.8	3.7	6.2	4.9	9.1
Succinic acid	0.2	0.9	0.7	0.7	0
Malic acid	0.2	0.4	0.9	0.9	0.7
Citric acid	0	0	0	0	0.6
Glyceric acid	4.5	20	19	19	11
Total in					
Sugar phosphates	14	19	18	15	15
Nucleotides	0	11	13	12	11
Maltose	9.8	57	66	53	52
Amino acids	120	45	49	40	40
Organic acids	5.7	25	27	25	21
Other substances	1.9	4.3	1.7	1.0	1.1
Soluble extract	150	160	170	150	140

metabolism differs from that of the other four anti-inflammatory compounds.

The most prominent effects of butazolidine, chloroquine diphosphate, cortisol and dexamethasone observed in the present work were concerned with the distribution of ^{14}C in the soluble intermediates of rat liver incubated with [^{14}C]glucose. The principal changes were a greatly reduced incorporation of labelled carbon into alanine and an increased accumulation of radioactivity in lactate, phosphoglycerate, glycerate, maltose, glutamate and nucleotides. [^{14}C]Alanine was the major component of the soluble intermediates of the liver when incubated in the absence of the drugs, which suggests that transamination is an important mechanism for the further metabolism of pyruvate derived from the labelled glucose used as substrate. Thus one possible explanation of the results reported above is that the four anti-inflammatory drugs interfered with this transamination reaction, thereby decreasing the incorporation of radioactivity into alanine and increasing the metabolism of pyruvate by other pathways. Alternative mechanisms are that either the utilization of the radioactive alanine is stimulated by the drugs or the ^{14}C in the substrate or in one or more of the metabolic intermediates is diluted by endogenous material, e.g. that derived from glycogen or protein breakdown, produced in the presence of the drugs. Such effects could have resulted in increased

amounts of isotope in lactate by the lactic dehydrogenase reaction, in phosphoglycerate and glycerate due to an accumulation of glycolytic intermediates, and in glutamate by an increased entry of the pyruvate carbon atoms into the tricarboxylic acid cycle. The increased accumulation of radiocarbon in the maltose fraction may be associated with the stimulation of liver-glycogen synthesis by cortisol and other steroids described by Winters & Morrill (1955) in whole animals, and by Chiu (1950) in slices: no information is available about similar effects of butazolidin or chloroquine on liver-glycogen formation. The significance of the increased incorporation of ^{14}C into the nucleotide fractions in the presence of the drugs cannot be assessed, since the intramolecular distribution of the isotope in these substances was not studied.

In contrast with the results obtained with liver and [^{14}C]glucose, the incorporation of radioactivity into alanine was not affected by any of the drugs if either [3- ^{14}C]pyruvate or [2- ^{14}C]acetate was used as substrate. Thus whatever may be the mechanism concerned with the decreased radioactivity found in alanine in the presence of the drugs, it possesses a drug sensitivity which varies with the labelled substrate. These findings suggest the possibility that there may be more than one metabolic pool of substances, such as pyruvate, which are concerned with alanine metabolism in liver cells.

SUMMARY

1. The effects of butazolidin, chloroquine diphosphate, cortisol and dexamethasone on the incorporation of ^{14}C from labelled substrates into the soluble intermediates of chopped preparations of rat liver and brain have been studied.

2. All the drugs increased the total incorporation of radioactivity from ^{14}C glucose in brain and decreased the total incorporation of the isotope from $[2\text{-}^{14}\text{C}]\text{acetate}$ in liver and brain. The total incorporation of ^{14}C from ^{14}C glucose and $[3\text{-}^{14}\text{C}]\text{pyruvate}$ in liver was not materially altered by the presence of the drugs.

3. The distribution of ^{14}C from ^{14}C glucose in the soluble intermediates of liver was affected by all the drugs. The main changes were a greatly reduced incorporation of the isotope into alanine and an increased accumulation of radioactivity in maltose, phosphoglycerate, glycerate, glutamate and lactate. None of the drugs affected the distribution of ^{14}C from ^{14}C glucose in brain, from $[3\text{-}^{14}\text{C}]\text{pyruvate}$ in liver or from $[2\text{-}^{14}\text{C}]\text{acetate}$ in both liver and brain.

4. Some implications of these results are discussed.

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Biosynthesis of Polynucleotides

4. THE UTILIZATION OF EXOGENOUS PRECURSORS BY *CANDIDA UTILIS**

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The presence of the enzyme polynucleotide phosphorylase in bacteria and in plant and mammalian tissues (Grunberg-Manago, Ortiz & Ochoa, 1956; Beers, 1957; Brummond, Staehelin & Ochoa, 1957; Littauer & Kornberg, 1957; Hilmo & Heppel, 1957; Hakim, 1959) has made it appear likely that nucleoside 5'-diphosphates are the immediate precursors in the biosynthesis of ribonucleic acid. Cell-free preparations have also been obtained which catalyse the incorporation of nucleoside 5'-triphosphates into ribonucleic acid (Cannellakis, 1957; Edmonds & Abrams, 1957; Hecht, Zamecnik, Stephenson & Scott, 1958; Chung & Mahler, 1958). Since nucleoside diphosphates and triphosphates are readily interconvertible in most tissues, it is difficult to distinguish between them as precursors of ribonucleic acid *in vivo*, or in tissue homogenates. However, it seems probable that incorporation of nucleoside 5'-monophosphates into ribonucleic acid

(Goldwasser, 1955; Heidelberger, Harbers, Leibman, Takagi & Potter, 1956) takes place through either the corresponding 5'-diphosphates or 5'-triphosphates. In part 1 of this series (Barker, Montague, Moss & Parsons, 1957) it was shown that, *in vitro*, pancreatic ribonuclease, besides catalysing the formation of ester linkages between simple nucleotides (Heppel, Whitfield & Markham, 1955), can also bring about the attachment of nucleotides to ribonucleic acid. Such a synthetic reaction taking place *in vivo* might manifest itself in the incorporation of nucleoside 3'- (or 2'-)phosphates into ribonucleic acid through the corresponding cyclic phosphates. It is clearly desirable to establish whether this biosynthetic route does in fact occur in tissues in any circumstances, and experiments are in progress to test this possibility. The work reported here is an attempt to determine the extent to which new internucleotide bonds of ribonucleic acid are formed by polymerization of nucleoside 5'-phosphates. In the present experi-

* Part 3: Barker & Foll (1957).