steroid utilization was noted[4,7]. Under the conditions of the experiments described here, steroid C-21 hydroxylase activity appeared to be maximal at about 50 μM progesterone (Fig. 3). However, the lack of responsiveness of this enzyme system to cyclic 3′,5′-AMP was not due to saturation with substrate. Thus, even at the highest substrate-level C-21 hydroxylase activity was stimulated about 35 per cent on addition of 0·1 ml. fresh rat serum to the incubation medium. Homologous serum has previously been shown to activate C-21 hydroxylation of added progesterone in adrenal homogenates[11] and microsomes[12]. In separate experiments, conversion of (4-^{14}C)-11-deoxycorticosterone to corticosterone was enhanced by cyclic 3′,5′-AMP (Fig. 4); 5′-AMP, the degradation product of 3′,5′-AMP, was only half as effective, ATP (2 mM) plus Mg^{++} (3 mM) also stimulated C-11β steroid hydroxylations in the presence of either substrate. This finding raised the possibility that adenyl cyclase was active in adrenal homogenates.

The selective stimulation of C-11β hydroxylations in adrenal homogenates by cyclic 3′,5′-AMP may have been brought about via enhancement of glucose-6-phosphate formation from glycogen and a resultant increase in NADPH generation. Although stimulation of NADPH generation in the adrenal cortex would be expected to accentuate all steroid hydroxylations, recent investigations from this laboratory[11] have shown that concentrations of NADPH-generating system in excess of the requirement for maximal production of corticosterone from progesterone selectively enhanced C-11β hydroxylation of this substrate. The mechanism of this phenomenon is not clear but may be related to a differential requirement for NADPH by mitochondrial C-11β hydroxylase and microsomal C-21 hydroxylase enzyme systems or to some functional association between the NADPH-generating system and adrenal mitochondria[11].

These observations may help in the elucidation of the mechanism of action of ACTH. A selective action of ACTH on C-11β hydroxylations in the adrenal cortex has been reported by Grant[13]. The investigations recorded here indicate that this selective effect of ACTH on steroid hydroxylations may be associated with rapid generation of NADPH in response to elevated levels of cyclic 3′,5′-AMP.

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Relationship between Pyrophosphate Content and Oxytetracycline Labelling of Bone Salt

HEATING of bone to 325° C has been shown to induce the formation of pyrophosphate[1–3]. The quantity of pyrophosphate so formed is greater in the epiphysis than in the diaphysis and greater in the younger than in the older animal, and is therefore presumably greater in newly laid down than in older bone salt[1]. The question to be investigated is whether pyrophosphate-yielding mineral of new bone has specific chemical characteristics. There is much evidence which suggests that the tetracyclines are deposited in high concentrations in newly formed bone salt[4]. This communication demonstrates that the tetracycline labels the form of the bone salt that yields pyrophosphate in large amounts after heating.

Two New Zealand rabbits were injected with 25 mg of oxytetracycline intravenously and killed 24 h later. The long bones of the hind quarters were excised and ground in a hand coffee mill. The resulting small particles were separated into two groups by means of ultra-violet light and a dissecting microscope. The particles that fluoresced brightly were picked out with the forceps and placed in one crucible while those that had no visible fluorescence were collected in another. Each crucible was heated to 325° C for 1 h in a muffle oven. The ortho- and pyro-phosphates obtained from these samples were separated by the method of François[5]. The resin used was 'Bio-Rad *AG* 1 × 8, 200–400 mesh'. Pyrophosphate was hydrolysed by heating for 30 min in 1 N hydrochloric acid at 100° C, and the orthophosphate was determined by the Fiske–Subbarow method[6]. The results, summarized in Table 1, clearly demonstrate that the fluorescent particles contain the mineral with the greatest potential for forming pyrophosphate. This corroborates the observation that both the tetracycline reactive and pyrophosphate-yielding minerals are found in less than 1 per cent of the bone salt that is the exchangeable, reactive, labile or metabolic fraction. The exact composition and structure of this fraction are not known, but the following are possibilities that have been considered in the literature: monetite, brushite, octacalcium phosphate, hydrated tricalcium phosphate, calcium-deficient apatite. New observations with new methods may be made using the foregoing method of labelling and separation of new mineral.

Table 1. PYROPHOSPHATE CONTENT OF BONE WHICH DOES AND WHICH DOES NOT BIND WITH OXYTETRACYCLINE

Sample	Rabbit (approximate age)	Pyrophosphate × 100 / ortho- + pyro-phosphate
Oxytetracycline-labelled bone	1–2 yr.	10·3
	6 mo.	15·8
Non-labelled bone	1–2 yr.	3·6
	6 mo.	5·0

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