in most of the animals, the thymus and Peyers' patches were entirely normal in appearance.

In the second group where the effect of stress was added to the action of the same dose of corticoids, extensive caryoclasia had taken place in the thymus and in the germinal centers of the Peyers' patches. In both tissues, massive and often confluent areas of pyknosis could be observed, their aspect remaining the same despite the type of stress to which the animals had been subjected.

In the third group, thymus caryoclasia was inhibited by the adrenalectomy, yet a very few areas of pyknosis could be observed in the Peyers' patches.

In the fourth group the classical picture of caryoclasia was observed in both lymphoid organs of the normal rats subjected to stress. It was noted however, that the caryoclasia was often less pronounced than it was in the second group of animals, particularly in those subjected to forced muscular exercise.

Comments. These experiments show that the influence of corticoids on the lymphoid system may be conditioned by non-specific stress. They militate in favor of the view that these hormones act on the lymphoid cells by some indirect means as well as imply that some conditioning factors related to the stress (enzymes?) influence this action.

Summary. The combined action of a small dose of cortical extract and non-specific stress of moderate intensity has a well marked caryoclastic effect on the lymphoid system of adrenalectomized rats, whilst the same dose of extract given to adrenalectomized, non-stressed rats, produces a negligible effect.

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Demethylation of C^{14}-Labelled Codeine in the Rat.* (17696)

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The demethylation of codeine has been postulated by Sanfillipo(1) on the basis of the presence of typical morphine withdrawal symptoms in dogs after the prolonged administration of codeine. More direct evidence was obtained by Bernheim's *in vitro* studies which showed that codeine after incubation with rat liver slices gave rise to a phenolic-OH compound, presumably morphine (2). Since Bernheim has also shown that morphine itself is oxidized at an appreciable rate, the extent to which demethylation occurs is presumably greater than that indicated by quantitative tests for residual morphine. Our experiments with radiocodeine (codeine*) now establish by direct evidence that codeine is demethylated in rats at the 3-position. The present studies were initiated by the observation that $C^{14}O_2$ rapidly appeared in the expired air of rats after administration of codeine labelled with C^{14} in the methoxy group. The present data include measurements of $C^{14}O_2$ output *in vivo* and analyses of tissues for $C^{14}O_2$-$HC^{14}O_3^-$ concentrations in addition to studies on the ability of various tissues to demethylate codeine* *in vitro*.

Methods. The animals used were adult, male and female albino rats of the Slonaker-Wistar strain maintained on a diet of Friskies dog food and tap water. The codeine* was synthesized by Chang, *et al.*(3), and had a specific activity of 0.9 microcuries/mg. The

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C^{14} was determined by the method of Dauben, *et al.*(4).

In vivo studies. The animals received a subcutaneous injection of 40 mg codeine* per kilo into the left leg and were placed in a glass metabolism cage. Expired CO_2 was collected in NaOH towers for periods of 1-31 hours and analyzed for C^{14}. For tissue analyses of radioactive CO_2-HCO_3^-, ether was administered to one rat 2 hours after codeine* administration, and a blood sample was obtained from the abdominal aorta. After death by exsanguination, the organs were removed and immediately placed in Potter homogenizers containing 0.5 N NaOH. Aliquots of the homogenate were acidified in the apparatus used for the *in vitro* studies, and the CO_2 collected for the determination of C^{14}. The residue after acidification was dried at 60° and combusted for the determination of the remaining C^{14} by the method of Van Slyke and Folch(5) as modified by Skipper, *et al.* (6).

In vitro studies. Rats were killed by decapitation, and the required organs were removed rapidly and placed in a cold box(7). Liver and kidney slices were prepared with a Martin slicer(8); lungs, spleen, brain, intestine and muscle (diaphragm) were cut into strips with a scalpel. Blood was drawn from the abdominal aorta into a heparinized syringe. Incubation of all tissues took place in an apparatus of the type described by Entenman for the wet oxidation of tissues(9).

The tissue slices were suspended in an Erlenmeyer flask containing 20 ml of Krebs-Ringer phosphate buffer(10) per g of tissue, with 0.2% glucose and 630 μg codeine* added. In the case of the experiments with blood, the Krebs-Ringer solution was omitted, the codeine* being added directly. Oxygen saturated with water vapor was admitted throughout the experiment, and the CO_2 produced was absorbed in a sodium hydroxide tower. The incubation flask was immersed in a water bath at 37°. At the end of 2 hours 2 ml of 1 N HCl were added to the flask in order to stop the action and decompose any bicarbonate formed. In the liver slice experiments a control flask in which HCl was added at the beginning of the experiment was set up under the same conditions. At the end of the experiment the incubation fluid was separated from the tissue slices by filtering through glass wool. The fluid was analyzed for C^{14} as well as for basic amines by the methyl orange technique of Brodie(11). The latter method was appraised for specificity by comparing codeine

TABLE I.
In vivo Formation of $C^{14}O_2$ from Codeine*

Rat No.	Time after inj.	Mg codeine* appearing as CO_2	% dose inj.
1	2 hr	2.05	22.2
3	" "	1.14	19.1
4	" "	1.12	14.1

* Labelled radioactive compound.

TABLE II.
Tissue Distribution of $C^{14}O_2$ and $HC^{14}O_3^-$ Resulting from Demethylation of Radio-codeine After Codeine* Administration.

Tissue	% dose inj.	Ratio C^{14} as CO_2-HCO_3^- / Total tissue C^{14}
Liver	.23	.034
Blood	.20	.091
Intestine	.13	.046
Fat	.06	—
Urine	.04	.012
Kidneys	.03	.038
Stomach	.03	.011
Brain	**.02**	**.067**
Spleen	.02	.043
Ovaries	**.02**	**.019**
Lungs	.01	.028
Heart	.003	**.031**

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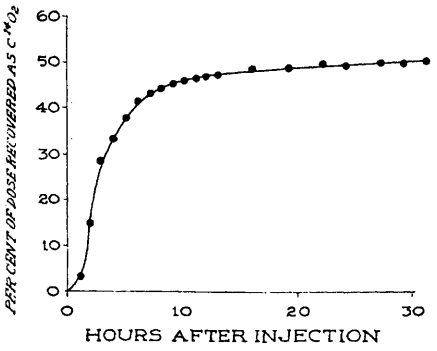

FIG. 1.

Recovery of $C^{14}O_2$ in expired air following injection of methoxy-labelled codeine.

distribution ratios between benzene and phosphate buffers of pH values 7.35, 7.12 and 6.72 respectively.

Results. In vivo studies. Approximately one-fifth of the radioactivity administered as a subcutaneous injection of 40 mg/kg codeine* appeared as $C^{14}O_2$ in the expired air within 2 hours (Table I). A small additional amount, less than 1% of the injected dose, was found distributed as $C^{14}O_2$-$HC^{14}O_3^-$ in the tissues at this time. Furthermore, analyses of the individual tissues indicate that the contribution of such radioactivity to the total activity of the tissue is minor (Table II). Thus, even in the case of the blood the C^{14} due to $C^{14}O_2$-$HC^{14}O_3^-$ is less than one-tenth of the total C^{14} content of that tissue. A detailed analysis of $C^{14}O_2$ excretion over a 31 hour period is presented in Fig. 1. A total of 51% of the radioactivity administered (as codeine*) was excreted during this period, the greatest part of this coming off during the first 6 hours. In this animal, peak $C^{14}O_2$ production occurred 3 hours after the subcutaneous injection.

In vitro studies. No demethylation was observed in our experiments (2 each) with blood and slices of lung, spleen, brain, intestine and the control liver experiment. Although both kidney and muscle exhibited a definite but limited capacity for demethylation, by far the most important site for this process was the liver (Table III).

Analysis of residual codeine (after incubation with liver slices) by the methyl orange technique showed good agreement with results obtained by analyzing for C^{14} (Table IV). Thus, the production of $C^{14}O_2$ *in vitro* is probably the result of demethylation rather than transmethylation. The codeine remaining was further identified by an appraisal of the specificity of the methyl orange technique for codeine in the manner described by Brodie. The solubility characteristics of this remaining codeine were found to be essentially the same as those of a standard codeine solution.

Discussion. Our *in vitro* experiments indicate that the production of $C^{14}O_2$ from codeine* labelled in the methoxy position is not a result of transmethylation. We have not definitely ruled out the possibility that the process is one of de-methoxylation. However, since Bernheim has shown that as much as 13% of codeine incubated with liver slices appeared as a phenolic-OH compound, it is probable that the process concerned is actually that of demethylation. It has been shown that codeine* is rapidly demethylated in rats *in vivo*. Approximately 20% of the injected C^{14} appears as expired CO_2 within 2 hours. The figure representing the portion of the effective dose demethylated is presumably

TABLE III.
In vitro Formation of $C^{14}O_2$ from Codeine*

Tissue	Time of incubation, hr	Codeine* identified as CO_2 in mg/kg fresh tissue	% of total codeine* added
Muscle	2	.33	.02
"	"	.48	.02
Kidney	"	1.33	.2
"	"	4.44	.4
Liver	"	202.	32.0
"	"	350.	43.4
" †	"	38.8	6.2

† This liver presented a gross aspect of chronic passive congestion.

TABLE IV.
Comparison Between C^{14} and Methyl Orange Methods of Determining Codeine Content of Filtered Incubating Fluid.

Sample	C^{14} analysis μg codeine*	Methyl orange analysis μg codeine
Control liver	603	575
Incubated liver	350	324
" "	185	182
" "	217	207

higher since within this period some 18% of the injected dose has not yet been mobilized (12). The methoxy group is oxidized to CO_2 and promptly excreted in the expired air. It is of interest to note that in spite of the large quantities of $C^{14}O_2$ present in the expired air, only negligible amounts are found in the tissues as CO_2 or HCO_3^-. One may assume, therefore, that in experiments dealing with the distribution of codeine* labelled in the methoxy position, the presence of C^{14} due to CO_2-HCO_3^- in the tissues does not modify the interpretation of results based on a consideration of the total C^{14} content of the tissue. This does not preclude the possibility that other radioactive compounds may have been formed or that modifications of the codeine* molecule may occur which contribute to the radioactivity of the tissue.

Demethylation *in vivo* proceeds at an appreciable rate for at least 6 hours after drug administration, at which time about 40% of the injected dose has been demethylated. As long as 31 hours after such an injection, a small but measurable amount of radioactivity is still being excreted as CO_2.

Although the *in vitro* experiments indicate that demethylation can take place to a small extent in kidney and in muscle, these organs probably do not play a significant role in this process *in vivo*. Considering the fact that in the body the total weight of muscle may be 10 times the weight of liver, and that the capacity for demethylation of the former is only 1/500 the latter, the over-all contribution of muscle would still be only 2% that of liver. Similar calculations for kidney show that *in vivo* this organ has a capacity amounting to 0.2% that of the liver. Thus, with approximately 98% of the demethylation occurring in the liver, one may assume that the appearance of radio-CO_2 in the expired air is due to demethylation of codeine* in the liver. On this assumption calculations of the *in vivo* capacity on a per kg wet liver basis give figures of, respectively, 240, 174 and 220 mg codeine* demethylated in 2 hours for the animals listed in Table I. These figures are of the same order of magnitude as those found in our *in vitro* experiments indicating that the enzymatic systems involved operate efficiently *in vitro*. Although it has been demonstrated that blood serum is capable of deacetylating heroin(13), rat blood apparently has no effect on the methoxy group of codeine.

In one of our *in vitro* experiments the presence of unspecified liver damage (as reflected in a gross appearance of chronic passive congestion) markedly interfered with demethylation. It is of interest to speculate how such interference *in vivo* would modify the analgesic response to codeine. The question of whether the analgesia of codeine is due solely to its demethylated product is worthy of consideration. The analgesic effect of the intact molecule can be studied under conditions where demethylation is prevented, as in hepatectomized animals or possibly in animals with severe liver damage.

Summary. Radio-codeine is demethylated *in vitro* and *in vivo*, the labelled methoxy group being oxidized to $C^{14}O_2$ which is expired by the lungs. The site of the demethylation process is primarily the liver; 40% of the injected dose is demethylated within 6 hours. Transmethylation has not been demonstrated *in vitro*.

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