depots of serotonin differ from one another in many respects. Although total amount of serotonin in brain is small compared to that in the gastro-intestinal tract it is apparent that synthesis and metabolism of serotonin occurs much more rapidly in the central nervous system than it does peripherally. This may also be true for norepinephrine since levels in the brain also rise rapidly following administration of monoamine oxidase inhibitors(15). However, with catecholamines too, peripheral depots, such as those in adrenal gland are known to turn over very slowly(17). The rapid turnover of these amines in the central nervous system is indicative of an important function.

Serotonin in blood platelets is quite distinct from that in other tissues. Its turnover does not reflect synthesis and metabolism by platelets. The amounts of serotonin in this tissue depend on amounts released from other depots most probably the gastro-intestinal tract. This would explain increased platelet levels in malignant carcinoid. The turnover in platelets is, therefore, a function of uptake and platelet survival.

Summary. Using tryptophan-C^{14} and 5-hydroxytryptophan-C^{14} it has been possible to measure turnover of serotonin in a number of tissues in the rabbit. The measured half lives are: platelets and spleen, 33-48 hours; stomach, 17 hours; intestine, 11 hours. Although amounts of serotonin in brain were too small to permit isotopic measurement it was possible to estimate its turnover from the increase following administration of harmaline, an inhibitor of serotonin metabolism. Such estimates indicate a half-life in brain of the order of minutes.

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Effect of N-Acetyl-para-Aminophenol on Plasma Levels of 17-Hydroxycorticosteroids.* (23869)

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Hench(1) observed that rheumatoid arthritis patients experienced marked or complete temporary remissions of arthritis during bouts of spontaneous intercurrent jaundice. Although there was some correlation between extent of remission and serum bilirubin concentration, Hench was of the opinion that bilirubin was not responsible for remission of

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arthritis. It is now known that urinary bilirubin, in jaundiced patients with bilirubinuria, is present almost exclusively in the form of glucuronide(2), as are urinary adrenocorticoids to a large extent(3,4). It is conceivable that remissions of arthritis observed by Hench (1) may have been due, at least in part, to elevated adrenocorticoid levels arising from competitive inhibition by bilirubin of steroid-glucuronide formation in the liver. The elevated plasma levels of 17-hydroxycorticosteroids observed in patients receiving large doses of diethylstilbestrol(5) and of salicylates(6) may possibly be explained in terms of a similar inhibition since these substances also are glucuronide precursors. Thus, substances which readily undergo conjugation with glucuronic acid may inhibit steroid conjugation *in vivo* by competing with steroid for available glucuronide-forming enzyme system. Should this be so, one would expect to find, upon administration of a steroid along with non-steroid glucuronide precursor, an elevated free steroid plasma level. N-Acetyl-para-Aminophenol (NAPA), when administered to humans, appears in urine in conjugated form to 85-96%, mainly as glucuronide and partly as ethereal sulfate(7,8). Single 2 g doses, administered orally to humans, caused 200% increase in urinary glucuronide output(9). In rabbits, it was more than 3 times as effective as acetylsalicylic acid as regards its ability to stimulate output of urinary glucuronide (9). The present paper is concerned with the effect of NAPA on plasma levels of free 17-hydroxycorticosteroids (17-OHCS) arising from exogenous hydrocortisone (cortisol) and prednisolone (Δ^1-cortisol) administered to rabbits.

Materials and methods. Female albino rabbits weighing 3 to 4 kg were used. Plasma levels of free 17-hydroxycorticosteroids (17-OHCS) were determined 1 or 2 hours after a single dose of cortisol or prednisolone, as indicated in the Tables. After 72 hour interval the steroid and NAPA were administered concurrently and plasma 17-OHCS again measured. (24 hours after administration of a single dose of cortisol or prednisolone there was no detectable 17-OHCS in plasma and hence no carry-over from one experiment to the next.) NAPA was administered by mouth as solution or suspension in tap water. Cortisol and prednisolone were dissolved in 50% propylene glycol, and injected intraperitoneally 30 minutes after NAPA had been given. Blood samples were obtained by cardiac puncture into heparinized syringes. Plasma was separated by centrifugation, and samples were stored in deep freeze until used for 17-OHCS determination. Plasma 17-OHCS were determined by the Silber-Porter method(10), as modified by Peterson *et al.*(11), 2 ml of plasma being used generally for each determination. Circulating 17-OHCS bearing a free Δ^4-3-oxo group were determined by the INH reaction of Umberger(12); and total glucuronic acid by the method of Fishman *et al.*(13), with the exception that pigment produced in the color reaction was extracted with n-butyl acetate in place of toluene.

Results. NAPA (250 mg/kg body weight), administered concurrently with cortisol (25 mg/kg), produced a marked increase in free 17-OHCS plasma levels, as measured by Porter-Silber method (Table I). At 1 and 2 hour intervals after dosage, the mean plasma level of 17-OHCS, in the presence of NAPA

TABLE I. Effect of N-Acetyl-p-Aminophenol (NAPA) on 17-OHCS* Plasma Levels in Rabbits, following Administration of 25 mg/kg of Hydrocortisone, Intraperitoneally.

Hr after dosage	17-OHCS plasma levels: Without NAPA, μg/ml	With NAPA,† μg/ml	% of control
1	5.25	9.30	177
1	2.20	4.20	191
1	3.90	10.20	262
1	6.40	10.10	158
1	4.40	9.40	214
1	2.30	9.70	422
1	3.20	7.10	222
‡	3.95 ± .24	8.56 ± .87	235 ± 34
2	4.97	11.15	224
2	5.81	13.13	229
2	4.68	17.62	376
2	5.44	13.87	255
2	6.94	12.23	176
2	5.42	13.89	256
‡	5.54 ± .32	13.68 ± .9	253 ± 27.3

* Determined by Peterson modification(10) of Porter-Silber method(9).

† N-Acetyl-p-Aminophenol (Atasol, Horner), 250 mg/kg body wt administered orally 30 min. before hydrocortisone.

‡ Mean ± S.E.

was 235 ± 34 and 253 ± 27%, respectively, of that attained when cortisol alone was given. A similar increase was obtained at cortisol dosage of 10 or 50 mg/kg and NAPA at 250 mg/kg.

No detectable amount of 17-OHCS was found in plasma of untreated rabbits or those administered an oral dose of NAPA alone. These results are in agreement with those of Done *et al.*(14), who found no circulating 17-OHCS in rabbit plasma even after ACTH stimulation. In this connection, Bush(15) found corticosterone to be the major adrenal steroid present in rabbit plasma. It is probable, therefore, that the 17-OHCS present in plasma of cortisol-treated rabbits was contributed entirely by exogenous cortisol.

It may be supposed that increased plasma levels of Porter-Silber reactive materials may consist predominantly of tetrahydro derivatives of cortisol rather than Δ^4-3-ketocorticosteroids. To obtain information on this point the isoniazid (INH) reaction of Umberger(12) was utilized. This reaction involves the formation of isonicotinylhydrazones of Δ^4-3-ketocorticosteroids, which can be measured colorimetrically, and the reaction has been shown to be specific for the Δ^4-3-keto group. The amounts of free Porter-Silber and INH-reactive materials were determined in aliquots of plasma from rabbits treated with cortisol alone and with cortisol plus NAPA. In each case the values for INH-reactive materials were essentially the same as those obtained with the Porter-Silber method, or only slightly lower. These results (Table II), indicate that increased plasma levels of 17-OHCS obtained after concurrent administration of cortisol and NAPA are comprised mainly of biologically active steroids, or steroids which are potentially so, since they contain the Δ^4-3-keto group.

TABLE II. Comparison between Porter-Silber and INH-Reactive 17-OHCS in Plasma, following Administration of 25 mg/kg Hydrocortisone Intraperitoneally.

Porter-Silber (17-OHCS), μg/ml		INH (Δ^4-3-KCS), μg/ml	
Without NAPA	With NAPA, 250 mg/kg	Without NAPA	With NAPA, 250 mg/kg
1.64	3.84	1.53	3.59
1.87	4.50	1.69	4.06
2.91	5.34	2.81	5.31
2.81	8.62	1.69	8.06
* 2.31 ± .32	5.57 ± 1.2	1.93 ± .3	5.25 ± .1

* Mean ± S.E.

TABLE III. Effect of NAPA on Δ^1-17-OHCS* Plasma Levels in Rabbits following Prednisolone Administration.

Prednisolone dosage, mg/kg	Δ^1-17-OHCS plasma level: Without NAPA, Conj.,‡ μg/ml	Without NAPA, Free, μg/ml	With NAPA† Conj., μg/ml	With NAPA† Free, μg/ml	With NAPA† Free, % of control
10	1.31	2.26	1.22	4.59	203
10	1.22	2.24	1.78	6.47	289
10		1.31		4.97	394
10		3.28		4.41	134
10		3.78		6.09	162
Mean ± S.E.		2.56 ± .43		5.31 ± .40	236 ± 46
25	3.09	5.34	4.69	10.12	189
25	3.28	7.50	4.89	12.37	165

* Determined by Peterson modification(10) of Silber-Porter method(9).

† 250 mg/kg of body wt administered orally 30 min. before prednisolone.

‡ Released by β-glucuronidase hydrolysis.

Table III shows the effect of NAPA on plasma levels of 17-OHCS arising from prednisolone (Δ^1-hydrocortisone) administration. Two hours after concurrent administration of prednisolone (10 mg/kg) and NAPA (250 mg/kg) the mean 17-OHCS plasma level was 236 ± 46% of the control value. In 2 rabbits when the prednisolone dosage was 25 mg/kg the circulating 17-OHCS in the presence of NAPA averaged 177% of the control level. While there was an apparent slight increase in plasma level of conjugated 17-OHCS, the principal effect of NAPA was to increase the plasma level of free 17-OHCS.

Due to the fact that prednisolone does not react with INH to form a hydrazone it was not possible to measure the Δ^4-3-keto-corticosteroid component of the plasma 17-OHCS following prednisolone administration. However, it is interesting that after administration of prednisolone, no INH-reactive material was present in plasma, thus providing further evidence that the first step in prednisolone metabolism is not its conversion to cortisol by reduction of the Δ^1-bond.

Discussion. The increased plasma levels of

free 17-OHCS attained by concurrent administration of cortisol or prednisolone and NAPA are, in all probability, due to the fact that NAPA provides an alternative substrate, in high concentration, to the glucuronide-forming mechanism in liver, thus inhibiting formation of steroid-glucuronide.

Although both Porter-Silber and INH-reactive 17-OHCS in plasma show a similar increase after NAPA administration, there is no evidence that the substances measured in each case are completely identical. The Porter-Silber reactive materials may consist partially of tetrahydro derivatives of cortisol, which presumably are biologically inactive. However, it is by no means certain that these materials are not potentially biologically active, provided that their rapid conjugation with glucuronic acid and subsequent excretion is prevented, thus providing an opportunity for reoxidation by the adrenals.

The marked increase in plasma INH-reactive 17-OHCS in the presence of NAPA is not consistent with the widely held view that corticosteroid conjugation with glucuronic acid takes place only after the Δ^4-3-keto group has been reduced to a saturated 3-hydroxyl group (16,3). In this connection Weichselbaum and Margraf(17) have presented evidence which suggests that conjugation may take place at carbon atom 21 without previous reduction at the Δ^4-3-keto group. They also found that following beta-glucuronidase hydrolysis of human peripheral plasma, Δ^4-3-keto-corticosteroids appeared in considerable amounts, as measured by the INH reaction. This could account for the marked increase in plasma INH-reactive materials caused by NAPA administration. These materials are biologically active, or potentially so, since they contain the Δ^4-3-keto group.

Of some interest is the fact that no INH-reactive material was present in plasma following administration of prednisolone. This indicates that the Δ^1-bond of prednisolone is not reduced to yield Δ^4-3-ketocorticosteroids. Studies in humans(18) have shown that no excretion of cortisol or cortisone occurs as a result of prednisolone administration, and Vermeulen(19) points out that in all metabolites of Δ^1-corticoids so far identified, the dienone grouping was retained. Sandberg and Slaunwhite(20) found that at least 40% of an administered dose of prednisolone was excreted as glucuronic acid conjugates. Although the conjugates could not be identified, conjugation presumably occurred at positions other than carbon 3. In this case, the increased plasma 17-OHCS effected by NAPA administration would be comprised of corticosteroids with the dienone structure intact.

The results presented here suggest the possibility that physiological effectiveness of endogenous or exogenous steroids may be greatly increased by retarding their inactivation through the use of competitive glucuronylation inhibitors. Such studies are now in progress at the clinical level. Preliminary results as to the effect of NAPA on 17-OHCS plasma concentrations are in agreement with those obtained in rabbits.

Summary. Administration to rabbits of N-Acetyl-p-Aminophenol (NAPA) in conjunction with cortisol or prednisolone resulted in a marked increase in plasma levels of 17-OHCS. This effect is presumed to be due to inhibition by NAPA of steroid-glucuronide formation in the liver.

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Cultivation in Tissue Culture of the Virus of Avian Lymphomatosis. (23870)

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The demonstration by Burmester, Prickett and Belding(1) that a cell-free extract of the Olson(2) transplantable lymphoid tumor could transmit avian visceral lymphomatosis, firmly established the fact that an agent or agents other than cells could induce the disease. Further studies(3) have shown that filterable agents are present in other rapidly growing lymphoid tumor strains. Because propagation of viruses in various cell-medium systems is now common, it was thought that a system might be found in which the agent of avian visceral lymphomatosis would multiply and show its presence by induction of cytological damage. Previous attempts to do this have been only partially successful. Doljanski and Pikovski(4) have reviewed previous work and reported that they were able to propagate the agent of fowl leucosis in cultures of common mesenchyme cells but were unable to produce cytopathological changes.

The present communication is a preliminary report on successful propagation from avian lymphomatosis liver filtrates of an agent producing focal lesions in tissue culture, serial passage of the agent, infection of embryos and chicks with the cultured agent, and recovery of the agent from the diseased embryos and chicks.

Materials and methods. Virus. The virus was contained in a filtrate of lymphomatous liver obtained from Dr. B. R. Burmester, Regional Poultry Research Laboratory, East Lansing, Mich. The filtrate was designated RPL-12-L28 and represented the 16th serial filtrate passage in White Leghorn chicks. *Tissue culture.* Chick embryo liver cultures were prepared as follows: Sixteen-day-old chick embryo livers were minced and mildly agitated with 0.85% trypsin solution. Gross particles that settled rapidly were discarded. The remaining cells were then sedimented by low-speed centrifugation and washed with balanced salt solution. Tubes were seeded with approximately 500,000 cells in 1 ml of medium consisting of 30% horse serum and 70% medium 199(5), and incubated 2 days at 37°C. The medium was then replaced with 100% medium 199, after which the tubes were inoculated with tissue filtrates or infective tissue culture fluid. Cultures of whole chick embryo were prepared in a similar manner from 10- to 12-day-old embryos. A slightly modified Eagle(6) medium containing 10% horse serum was used. After 24 hours' incubation at 37°C, the medium was changed to 100% 199, and the tubes were inoculated. *Neutralization tests.* Serum neutralization tests in tissue culture were done by inoculating tubes with 0.1 ml of serum-virus mixture. A constant amount of virus (1,000 to 10,000 tissue culture infectious doses) was mixed with serial 2-fold dilutions of serum, and the mixture remained at room temperature for at least 2 hours. The neutralization titer of the serum was the highest dilution of serum in the incubated inoculum which prevented cellular destruction for 7 days.

Results. Tissue culture. Chick embryo liver cells grew on glass as discrete islands of hepatic cells surrounded by large strands of fibroblasts. On low power microscopic examination of living preparations, the first noticeable effect of the virus was swelling of he-