body assays. In addition, uniform preparations of formalin preserved cells were difficult to reproduce. As a result, other methods were investigated which would still retain the advantage of employing preserved cells in hemagglutination assays.

The glutaraldehyde method for preservation of red blood cells appeared to fulfill the desired requirements. A shorter time of treatment with the fixing reagent resulted in stable, uniform preparations of erythrocytes which were insensitive to freezing and thawing, and changes in pH and osmolarity.

Two antigens, human serum albumin and beta lactoglobulin, were covalently linked to these fixed cells by use of the bifunctional reagent, bis-diazotized benzidine. This method should, therefore, be useful for many types of antigens which contain diazotizable histidine and tyrosine residues.

During the course of these studies, it was noted that human serum albumin was absorbed to the glutaraldehyde-treated cells in the absence of any agent such as tannic acid or bis-diazotized benzidine. The absorption of proteins to glutaraldehyde-treated sheep erythrocytes is not a universal phenomenon, as bovine beta lactoglobulin was not absorbed by the same preparation of cells.

Employing BDB-BLG-glutaraldehyde-fixed cells and anti-BLG sera, the inhibition of hemagglutination was found to be a very sensitive assay for small amounts of BLG; 10^{-5} μg of BLG could be detected. More precision in this assay can be achieved by measuring the settling time of agglutinated *versus* non-agglutinated cells(5).

Summary. Methods have been described for the preservation of sheep erythrocytes by brief treatment with glutaraldehyde. These cells could be conjugated to proteins in the presence or absence of coupling reagents and were specifically agglutinated by specific anti-protein sera. These antigen-conjugated cells were stable for at least 6 months. Utilizing these cells, the inhibition of hemagglutination was found to be a sensitive technique for detection of small amounts of antigen.

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Effect of Steroids on Disposition of Oxyphenbutazone in Man.*† (31954)

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The administration of more than one drug at a time is common medical practice, and numerous examples of synergistic and antagonistic effects are known. Many of these effects are assumed to be interactions at the receptor sites. However, in recent years several instances have been reported in man and animals in which the apparent synergistic or antagonistic effects could be correlated with an alteration in the metabolic fate of one drug induced by the other(1,2). Some of these

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alterations have been associated with the "induction" or inhibition of microsomal drug metabolizing enzymes. The ability of some natural steroids to serve as substrates for these enzymes(3) makes it of interest to consider the effect of steroids on the fate of drugs.

The present study describes the effect of several steroids on the plasma levels of oxyphenbutazone, a non-steroid anti-inflammatory agent which is often used in combination with steroids. Oxyphenbutazone has a long half life in man, resulting in quite stable "plateau" plasma levels when the drug is administered daily in a fixed dosage pattern(5).

Methods. Plasma concentrations of oxyphenbutazone were determined by a previously published procedure(4). Oxyphenbutazone glucuronides were measured by a method based on hydrolysis with b-glucuronidase(6). All drugs were given orally to hospitalized subjects whose clinical states were essentially stable. The drugs employed were in the form of commercial tablets of oxyphenbutazone (Tandearil); methandrostenolone (Dianabol); prednisone (Meticorten); and dexamethasone (Decadron).

Two protocols were followed: 1) Oxyphenbutazone was administered in a consistent manner (300 or 400 mg/day) throughout the study. There was an initial control period after which a steroid was co-administered for several days. The steroid was then withdrawn while oxyphenbutazone administration continued for a final control period in some experiments. Blood specimens were drawn in the morning before dosage on appropriate days. 2) Blood levels were measured for 3 days after a single 600 mg dose of oxyphenbutazone in order to determine its half life. A month later the same subjects again received the same dose of oxyphenbutazone, but this time they were also treated with 2.5 mg b.i.d. of methandrostenolone for 4 days before and 3 days after the single dose of oxyphenbutazone.

Results. In 6 experiments, subjects were treated with oxyphenbutazone continuously for 2 to 5 weeks supplemented for 3 to 16 days with the anabolic steroid methandrostenolone 5 or 10 mg/kg. Blood levels of the drug were determined at intervals of one to three days just before the morning dose. The plasma levels of oxyphenbutazone during this period of steroid supplement were compared with those in the control period (Fig. 1 and Table I). In all but one instance blood levels

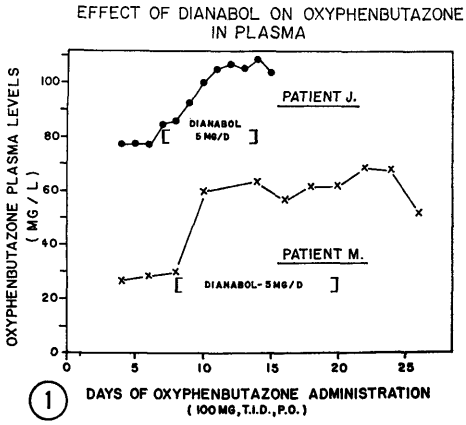

FIG. 1.

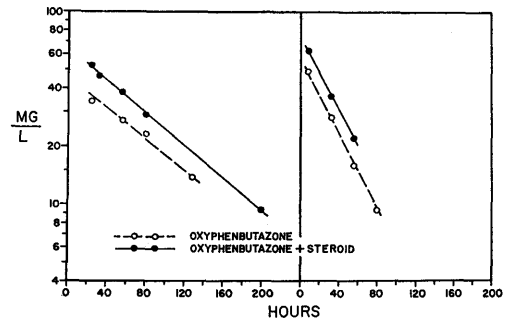

FIG. 2. Plasma concentration of oxyphenbutazone in 2 subjects following a single 600 mg dose of the drug orally, alone *vs.* in combination with methandrostolone 2.5 mg b.i.d. beginning 4 days before the oxyphenbutazone dose, and continuing throughout period of observation.

TABLE I. Effect of Methandrostenolone on Plasma Levels of Oxyphenbutazone.*

Subject	Range of levels (mg/l) Without steroid	During steroid	Avg % increase with steroid
M	27-30	45- 67	100
Ma	58-64	77- 84	35
A	53-62	76-115	55
Ro	56-67	67-115	30
J	77-85	86-109	35
Mc	70-95	85-100	5

* Subjects Ma, R, and J received 5 mg/day of steroid, the others 10 mg/day. Subjects Ma and Ro received 100 mg t.i.d. of oxyphenbutazone, the others 200 mg b.i.d.

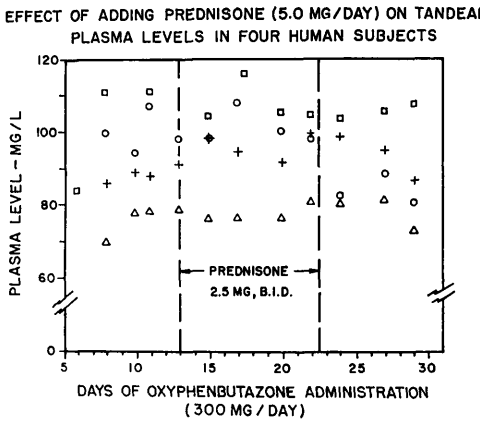

FIG. 3.

of oxyphenbutazone increased over 30% on addition of methandrostenolone.

Oxyphenbutazone blood levels were determined twice in each of 2 subjects following a single 600 mg dose with and without concomitant methandrostenolone administration. At every point the drug level was higher in the experiment with steroid than without steroid. However, the rate of drug disappearance after the first few hours was not altered by methandrostenolone (Fig. 2), *i.e.*, the slopes of the disappearance curves are the same.

In contrast to the effect of this anabolic steroid, the glucocorticoid prednisone, in a dose of 5 mg/day, had no influence on oxyphenbutazone levels (Fig. 3 and Table II). Two of the subjects who responded to methandrostenolone were also in this group.

Another glucocorticoid, dexamethasone, also failed to alter the plasma level of oxyphenbutazone in two subjects (Table III).

TABLE II. Lack of Effect of Prednisone on Plasma Levels (mg/l) of Oxyphenbutazone.*

Subject	Control period	On prednisone	Off prednisone
N	102 (84-111)	108 (104-127)	106 (103-107)
L	102 (94-107)	101 (98-108)	84 (80- 88)
R_0	88 (86- 91)	93 (91- 98)	94 (86- 98)
R_1	76 (70- 78)	77 (76- 81)	78 (72- 81)
M	46 (41- 58)	50 (42- 53)	50 (49- 56)

* Figures represent average (range in parentheses) levels during period indicated (minimum of 3 values per period determined every 2 or 3 days). Throughout the study all subjects received oxyphenbutazone (200 mg A.M. and 100 mg P.M.) daily, and 2.5 mg prednisone b.i.d. as indicated.

Discussion. The initial observations suggested that methandrostenolone might interfere with the metabolism of oxyphenbutazone. However, the effect of the steroid on the disappearance rate of a single large dose of the drug indicated that the steroid altered the distribution of oxyphenbutazone between plasma and tissues, rather than its rate of metabolism. This observation is consistent with the finding in one subject that there was no change in urinary excretion of oxyphenbutazone glucuronide(6) upon co-administration of methandrostenolone. An increase in the intestinal absorption of oxyphenbutazone is not involved, since the drug is completely absorbed(7).

TABLE III. Effect of Decadron on Oxyphenbutazone Plasma Levels During Continuous Administration of Oxyphenbutazone, 300 mg/Day.

Day	Plasma oxyphenbutazone levels, mg/l Subject C	Subject H
8	101	85
11	118	74
14	89	65
18*	89	59
20*	102	56
22*	96	62
25	83	59
27	—	53
29	93	60
32	79	61

* Decadron 0.75 mg b.i.d. was administered from day 14 to day 22. All blood specimens were drawn in the morning before dosage.

The nature and mechanism of methandrostenolone induced alteration in the apparent volume of distribution of oxyphenbutazone is not yet known. The relationship of this phenomenon to the reported effect of methandrostenolone on lowering the doses of corticosteroids required in rheumatoid subjects(8) or the efficacy of small doses of phenylbutazone administered with methandrostenolone(9) also remains to be determined.

Summary. The anabolic steroid methandrostenolone causes an elevation in oxyphenbutazone plasma levels in man. The elevation is not due to a slowed rate of metabolism, but to an altered distribution between plasma and tissues. The glucocorticoids, prednisone and

dexamethasone, do not influence the blood levels of oxyphenbutazone.

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Recovery of an Adenovirus from a Feral Rodent *Peromyscus maniculatus.** (31955)

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During an intensive search for arbovirus infections in small mammals from Kern County, California, an apparently new adenovirus was recovered from the blood of a feral rodent (*Peromyscus maniculatus*, the white footed deer mouse). This report describes the circumstances surrounding the isolation and the characteristics of this virus.

From November, 1960, through April, 1963, blood samples were collected from 1,890 small wild mammals, representing 19 species. All animals were live-trapped in rural areas of Kern County, either from agriculturally developed farmlands or from desert habitats adjacent to such environments. Blood samples represented a 0.3 ml aliquot diluted in 2.0 ml of a diluent comprised of 2% heparin (1:10,000) and 20% normal rabbit serum in physiologic saline. Samples were held at —70°C in sealed glass ampoules until tested.

The blood sample from each animal was inoculated (0.01 ml) into each of 8 suckling (2- to 3-day-old) mice by the intracerebral route and 0.1 ml into each of 2 tubes of 10-day-old hamster kidney cell cultures (HKCC) (1). Presence of virus was indicated by mice becoming ill or dying within a 21-day period or by observing cytopathogenic changes in cell cultures within a ten-day period.

Primary isolation. A virus (strain E-20308) was isolated from the blood of an adult female *P. maniculatus* trapped and bled on January 18, 1963, in a desert study area approximately 45 miles west of Bakersfield, Calif. Mice and HKCC were inoculated with this blood on May 9, 1963. Six of eight mice were dead the morning following inoculation and were discarded as presumably nonspecific deaths. A second litter of mice was inoculated with the original blood sample. On the second day postinoculation, 2 mice were dead and brains were removed from 2 additional mice that appeared to be abnormal. A fifth mouse was found dead on day 4 and the other 3 remained normal. Subpassage of homogenates of the 2 brains from sacrificed mice did not produce illness in suckling mice or cytopathogenic effects (CPE) in HKCC. The cells of HKCC inoculated with the original blood

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