TABLE I.
Sensitivity of the Normal and the *p*-Rosaniline Fast Strain of *T. brucei* Toward Different Anti Trypanosomal Agents.

Compound	Route	Minimal active dose (mg/kg)	
		Normal strain	Resistant strain
Neo-arsphenamine	subcut.	12.5	12.5
Mapharsen	"	1.0	1.0
Acriflavine	"	5.0	5.0
Emetic tartar	"	5.0	5.0
6,6′-ureylene-bis (4-amino-2-methyl-quinoline)	"	62.5*	62.5*
p-Rosaniline HCl	"	10.0	> 50.0
	per os	100.0	>500-1000.0

* Identical with the curative dose.

p-rosaniline-fast strains of *T. equiperdum** (PR I, PR III) which also lacked the parabasal body no longer responded to subcutaneous treatment with 500 mg of the quinoline derivative per kg, while a tenth of this dose (50 mg/kg) cured the infection with the parent strain.

These observations indicate that an atypical *p*-rosaniline-fast strain of *T. brucei* was obtained. It was characterized by the persistence of the parabasal body and the therapeutic sensitivity to 6,6′-ureylene bis (4-amino-2-methylquinoline). *p*-Rosaniline-resistant modifications of *T. equiperdum* exhibited as a rule the loss of both these properties. The question of the mechanism by which these 2 phenomena, namely, presence or absence of the parabasal body and sensitivity towards 6,6′-ureylene bis [4-amino-2-methyl quinoline] are related or whether they are related at all cannot be decided on the basis of our present experience. We are also not in a position to state definitely that other strains of *T. brucei* might give a similar response to *p*-rosaniline or even that the same strain of *T. brucei* will always react in the same atypical way to attempts of rendering it *p*-rosaniline-fast.

Summary. A *p*-rosaniline-resistant modification of *T. brucei* is described in which—contrary to the experience with other trypanosome strains—the parabasal body persisted. The strain also failed to show the overlapping resistance to 6,6′-ureylene [4-amino-2-methylquinoline] which could be demonstrated in *p*-rosaniline-fast strains of *T. equiperdum.*

* The experiments with *T. equiperdum* were carried out by Schnietzer in the Connaught Medical Research Laboratories of the University of Toronto.

16211

Stability of Natural Progesterone.*

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In 1936 approximately one gram of crystalline progesterone was isolated from sows' ovaries for use in the study of some of the biological properties of the hormone.[1] The major portion of this lot was used in the study of various aspects of the hormonal control of

* The authors wish to thank Miss Lila F. Knudsen, of the Food and Drug Administration, Washington, D.C., for her assistance in the statistical analysis of the data.

[1] Allen, W. M., and Goetsch, Carl, *J. Biol. Chem.*, 1936, **116**, 653.

pregnancy in rabbits. During that study no bioassays of the hormone were made to specifically test its stability. The compound was assumed to be stable since the physical characteristics of the crystals did not appear to change with the lapse of time. Small portions of the preparations obtained in 1936, however, were kept for bioassay at a later date to determine their stability. These samples of alpha and beta progesterone have now been assayed and have been found to be as active today, after storage for 10 years, as the first preparations obtained by Wintersteiner and Allen in 1934.[2]

The preparations recently subjected to bioassay were stored during the past decade in small, unsealed, glass-stoppered, vials. The vials were in turn subjected to the temperate environment of a desk drawer without any further attention until the samples were removed for the present experiment.

The melting points of the compound, after 10 years, were essentially the same as originally. The present sample of alpha progesterone was made up of the unused portions of several lots, the original melting points of which ranged between 126° and 129°C. The melting point obtained recently was 123°-124°C. The present sample of beta progesterone melted at 120°-121°C; the original lots from which the present small sample was obtained had melting points ranging from 118°-121°C.

Both the alpha and beta forms were bioassayed according to a slight modification of the original Corner-Allen method.[3,4] Our modified method differed from the original in that ovulation was induced in the rabbits used for the tests by employing commercial chorionic gonadotrophin rather than by mating the animals with the male. Ovulation was obtained by injecting intravenously a freshly prepared aqueous solution of powdered, dry, human chorionic gonadotrophin equivalent to approximately 20 international units. Laparotomy was performed 18 to 24 hours after the administration of the gonadotrophin. At the time of the operation, the ovaries were inspected and if ovulation had occurred, both ovaries were removed and the distal third of one uterine horn resected to serve as a histological control. Beginning on the day of operation and on each day thereafter for a total of 5 days, one-fifth of the total dose of progesterone was administered subcutaneously in 0.2 cc of sesame oil. Twenty-four hours after the last dose, the animals were sacrificed and the uteri removed and fixed in formalin. Transverse sections of the uterine horns were then prepared and stained with hematoxylin and eosin in the usual manner.

The degree of endometrial proliferation was determined by two methods: (a) by comparison with illustrations already published[4] and (b) by measuring the amount of proliferation with a planimeter. The planimetric measurements were made from tracings of the projected image of the section drawn on white paper in the following manner. Three circumscribing lines are drawn in different colors to locate the areas for measurement. Since degree of proliferation is determined as a ratio, the degree of magnification does not need to be kept constant. First, a line (M) is drawn which separates the myometrium from the endometrium. Next, a line is drawn which outlines the outermost aspect of the glands (S). This line divides the endometrium into an outer, unproliferated, non-glandular area and an inner glandular area. This line coincides with the line designated as (M) in some regions of the well proliferated uteri. Finally, a line (L) is drawn separating the lumen from the glands. These lines are separately traced with the planimeter and the circumscribed areas determined. The portion of the endometrium which has become glandular (R) is given by the formula:

$$R\ (\text{response}) = \frac{S - L}{M - L} = \frac{\text{area of glands}}{\text{area of endometrium}}$$

For the purpose of comparison, Table I is compiled from bioassay data obtained in 1934 using the original Corner-Allen method. The sections were re-evaluated without exact

[2] Wintersteiner, O., and Allen, W. M., *J. Biol. Chem.*, 1934, **107**, 321.

[3] Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, **88**, 326.

[4] Allen, W. M., *Am. J. Physiol.*, 1930, **92**, 174.

TABLE I.
Original Bioassay of Progesterone in 1934 (Corner-Allen Method).
Endometrial Proliferation.

Prep. No.*	Dose, mg	1934	1946†	R	Log dose × 10	θ
			Alpha Progesterone.			
1	1.77‡	4+	4+	.723	1.248	58.3
1	1.33	4+	3+	.816	1.123	64.6
2	1.22	4+	3+	.675	1.086	55.2
3	0.91	3+	3+	.502	.959	45.1
2	0.90	3+	3+	.696	.954	56.6
1	0.88	2+	2+	.544	.944	47.5
2	0.61	1+	1+	.292	.785	32.7
3	0.60	1+	1+	.218	.778	27.9
1	0.44	1+	2+	.313	.643	34.0
			Beta Progesterone.			
2	1.47	4+	4+	.802	1.802	63.6
3	1.28	4+	4+	.784	1.107	62.3
1	1.17	3+	4+	.767	1.068	61.1
2	1.05	2+	2+	.582	1.021	49.7
3	0.94	2+	1+	.402	.973	39.3
1	0.70	2+	3+	.588	.845	50.1
2	0.63	1+	1+	.304	.799	33.4
3	0.63	1+	2+	.357	.799	36.7

* Preparation numbers same as the number from Table I, *J. Biol. Chem.*, 1934, **107**, 321.
† Re-evaluation of the proliferation of the original sections.
‡ Data from animals receiving 1.77 and 1.33 mg of alpha and 1.47 of beta were excluded in the determination of the regression lines.

knowledge of the original interpretations. The estimates of the degree of proliferation of the endometrium were virtually the same as originally.

The bioassay of the samples of alpha and beta progesterone after storage for ten years is recorded in Table II. Several animals were used at each of several levels of dosage, spread over the critical range, to find out how much variation in response is to be expected.

Comparison of the data in Tables I and II shows, first of all, that the quantities necessary to produce full proliferation, and the quantities which produce little or no proliferation, are virtually the same. This indicates beyond doubt that there has been no great change in activity during the interim. Secondly, the variability of response in the critical range of dosage seems to be about the same.

As yet there is no generally accepted method for comparing the activity of compounds having progestational activity with the activity of the international standard of progesterone. Inspection of Tables I and II certainly indicates that the preparations obtained in 1936, and assayed in 1946, have approximately the same activity as the original preparations obtained and assayed in 1934. There is every reason for believing that the results should be similar, since the physical characteristics remained essentially unchanged during the storage period. More detailed comparison of the results of the bioassays, therefore, is justified as it affords in a sense an indication of the reliability of bioassays of identical compounds at widely separated periods of time, even though the results can only be compared with each other and not with concurrent assays of the international standard.

The best approximation of a linear relationship between the dose administered and the response of the endometrium (R—obtained by the planimeter) was secured when the dose was transformed to log dose and the angular transformation† was used on the response. The data for each of the four preparations were subjected to the above transformation and the dosage response curves fitted by the method of least squares. Only tests falling in the critical range (0.4-1.2 mg) were used. The results of this form of analysis of the data are recorded in Table III. The slopes are not very steep, but in all cases they are statistically significant, since in each case the slope

† Fisher, R. A., and Yates, F., *Statistical Tables for Biological, Agricultural, and Medical Research*, Table XII, p. 42, Oliver and Boyd, Edinburgh, 1938.

TABLE II.
Bioassay of Progesterone After Storage for 10 Years (Modified Corner-Allen Method). Endometrial Proliferation.

Dose, mg	Proliferation, Avg	R, Avg	Log dose × 10	θ
		Alpha Progesterone.		
1.6*	4+	.702	1.204	56.9
1.4	4+	.536	1.146	47.1
1.4	4+	.651	1.146	53.8
1.4	4+	.837	1.146	66.2
1.2	3.5+	.741	1.079	59.4
1.2	3.5+	.708	1.079	57.3
1.2	3.5+	.805	1.079	63.8
1.0	2.5+	.650	1.000	53.7
1.0	2.5+	.646	1.000	53.5
1.0	3.5+	.664	1.000	54.5
.8	2.5+	.632	.903	52.6
.8	3.5+	.620	.903	51.9
.8	[illegible]+	.581	.903	49.7
.8	2[illegible]	.508	.903	45.5
.6	2+	.457	.778	42.5
.6	+	.406	.778	39.6
.6	2+	.461	.778	42.8
.6	+	.373	.778	37.7
.4	0	.182	.602	25.2
.4	+	.450	.602	42.1
.4	+	.476	.602	43.7
.4	+	.433	.602	41.2
.2	0	.143	.301	22.2
.2	0	.193	.301	26.0
.2	0	.158	.301	23.4
		Beta Progesterone.		
1.2	3+	.691	1.079	56.3
1.0	3+	.614	1.000	51.6
1.0	3.5 +	.714	1.000	57.7
.8	1+	.330	.903	35.1
.8	4+	.761	.903	60.8
.8	2+	.541	.903	47.4
.8	2+	.488	.903	44.3
.6	3+	.632	.778	52.6
.6	5+	.273	.778	31.5
.4	+	.438	.602	41.5
.4	0	.026	.602	9.2

* Data from animals receiving 1.6, 1.4, and 0.2 mg were excluded in the determination of the regression lines.

TABLE III.
Analysis of the Dosage Response Curves (log dose — θ response).

Preparation	No. of animals	Intercept of regression line	Standard error of estimate	Slope	Standard error of slope
1934 Alpha	7	50.5	6.02	64.5	15.2
1934 Beta	7	51.6	6.54	73.4	19.1
1946 Alpha	18	54.4	4.69	46.6	6.4
1946 Beta	11	53.8	10.67	66.9	20.7

is more than 3 times its standard error. Likewise the values for the intercept at a dose of 1.0 mg show very little variation. The data indicate, therefore, that the dosage response curves from the data of 1934 do not differ significantly from those obtained from assay in 1946 of preparations that were 10 years old. Likewise there is no significant difference between the curves for alpha and beta progesterone. Using a biological assay procedure with the 1934 data labeled "standard" and the 1946 data labeled "unknown," the potency

thus obtained does not differ significantly from 100%.

Summary. Natural progesterone isolated from pigs' ovaries in 1936 shows no loss of activity after storage for 10 years in glass-stoppered vials at room temperature. Essentially similar methods of bioassay at widely separated intervals of time give dosage response curves that show no statistically significant differences.

16212

Relation of Complement to Blood Coagulation.

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Evidence has been presented both for and against the participation of complement in the coagulation of the blood. Wadsworth, Maltaner and Maltaner[1] favored the affirmative view because many substances were found to have both anticoagulant and anticomplementary actions. Maltaner[2] reported a close correlation between thromboplastic and complement-fixing power of tissue extracts and sera. On the other hand, Wilander[3] and Ecker and Pillemer[4] found great quantitative differences between the relative anticoagulant and anticomplementary properties of a number of substances. Quick[5] showed that complement as a whole is not the same as prothrombin. However, much recent work points to the existence of an additional component of the coagulation system, concerned with the conversion of prothrombin to thrombin. We[6] have already quoted much of this work but should mention in addition the contributions of Ware, Guest, and Seegers[7,8] and the extensive and convincing investigations of Owren.[9] A study of the role that complement plays in the conversion of prothrombin to thrombin appears timely.

Methods. Human plasma was freed of complement activity in 3 ways: by aging and by treatment with zymin and with ammonia. After aging for 2 to 3 months at icebox temperature plasma had no complement activity. Zymin powder was prepared from yeast essentially as described by Ecker, Jones, and Kuehn.[10] Two hundred and fifty milligrams of zymin were mixed with 1 cc of oxalated plasma and 1 cc of imidazole buffer at pH 7.2. Ordinarily serum is incubated with zymin for one hour at 37°C in order to inactivate complement, and smaller quantities than used by us suffice. We found that if the plasma was centrifuged immediately after thorough mixing with zymin, most but not all of the complement activity had disappeared by the time the supernatant was tested, about 20 minutes after the addition of zymin. The remaining complement activity disappeared gradually over a period of one to 2 hours in the absence of zymin. This was the procedure followed in preparing the zymin plasma used in the experiments described subsequently. This

1 Wadsworth, Augustus, Maltaner, Frank, and Maltaner, Elizabeth, *J. Immunol.*, 1937, **33**, 297.

2 Maltaner, Frank, PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 302.

3 Wilander, Olof, *Skandinav. Arch. f. Physiol.*, 1938, **81** (Suppl. 15), 89 pp.

4 Ecker, E. E., and Pillemer, L., *J. Immunol.*, 1941, **40**, 73.

5 Quick, A. J., *J. Immunol.*, 1935, **29**, 87.

6 Mann, F. D., Hurn, Margaret, and Magath, T. B., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 33.

7 Ware, A. G., Guest, M. M., and Seegers, W. H., *Science*, 1947, **106**, 41.

8 Ware, A. G., Guest, M. M., and Seegers, W. H., *J. Biol. Chem.*, 1947, **169**, 231.

9 Owren, P. A., *The Coagulation of Blood; Investigations on a New Clotting Factor*, Oslo, J. Chr. Gundersen, Boktrykkeri, 1947, 327 pp.

10 Ecker, E. E., Jones, C. B., and Kuehn, A. O., *J. Immunol.*, 1941, **40**, 81.