arches, and massiveness of the dentition. In no character was there a wide divergence between these two animals. On the other hand, the Peruvian fossil differs from the illustrations of *C. gezi* and *C. nehringi* in the shape of the dorsal profile of the skull. In *C. gezi* and *C. nehringi* the frontals are inflated, as in many domestic breeds of dog. None of the La Brea specimens from Peru show this inflation. The rostrum is shorter and lighter in both *C. gezi* and *C. nehringi* than in *C. dirus* and is so constructed the P^4 seems to be set below and behind P^3 to give the suggestion of a "step down" from P^3 to P^4. This condition is not clearly marked in *C. dirus*.

Comparison of the postcranial skeleton of *C. dirus* and that of the Peruvian fossil canid shows a striking similarity both in shape and dimensions, while the postcranial material of both *C. gezi* and *C. nehringi* is so scarce that little comparison is possible.

The conclusion resulting from this first investigation is that a large wolf-like creature existed in the Pleistocene of Peru and that this creature belongs within the genus *Canis* and is closely related to *C. dirus* of the Californian Pleistocene tar pits. Until further material has been developed from the matrix it is too early to state definitely whether it is identical with *C. dirus* or not, and it is proposed for the present to refrain from giving the Peruvian wolf a separate name, since its synonymity with *C. dirus* is a distinct possibility.

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Effect of Anxiety on the Akerfeldt Test

Abstract. Acute anxiety episodes do not significantly alter the lag time of the Akerfeldt test, serum ceruloplasmin level, or serum ascorbic acid level in man.

Ostfeld, Abood, and Marcus have recently reported that the concentration of ceruloplasmin in the serum of "disturbed" patients is significantly greater than the levels found in more "tranquil" patients (*1*). The primary behavioral characteristic of the disturbed patients which differentiated them from the less disturbed group was their greater degree of anxiety. We have attempted to verify this claim by experimentally raising the anxiety level under hypnosis of a group of normal volunteers and measuring their ceruloplasmin level (along with several related biochemical variables) before, during, and after the experimental anxiety state. A detailed description of the experimental subjects and the mode of their selection, the experimental design employed, the method of producing the experimental anxiety state during the hypnotic trance, and of the anxiety state achieved, has been reported elsewhere (*2*).

Table 1. Lag times and slopes of Akerfeldt tests and serum ascorbic acid levels of 12 normal subjects on four different occasions. (Means ± standard deviations for six male and six female subjects.)

Occasion	Assay					
	Lag time (sec)		Slope (ΔO.D./sec)		Ascorbic acid (mg/100 ml)	
	M	F	M	F	M	F
Pre-hypnosis	167 ± 162	318 ± 118	8.2 ± 3.1	11.2 ± 3.5	1.39 ± 0.97	2.70 ± 0.70
Hypnosis	163 ± 161	317 ± 153	7.9 ± 2.9	11.6 ± 3.2	1.24 ± 0.88	2.53 ± 0.67
Hypnosis + anxiety	163 ± 156	285 ± 79	8.7 ± 2.3	11.5 ± 3.4	1.28 ± 0.76	2.67 ± 0.66
Post-hypnosis	167 ± 162	302 ± 129	10.4 ± 7.4	12.6 ± 4.1	1.29 ± 0.72	2.60 ± 0.48

Table 2. Analysis of variance among the lag times, slopes, and ascorbic acid levels by sexes and occasions.

Variation	df	Lag time		Slope		Ascorbic acid	
		MSq.	F	MSq.	F	MSq.	F
Between sexes	1	236,602	2.97	100.34	2.98	21.081	10.44*
Between subjects in sexes	10	79,722		33.63		2.020	
Between occasions	3	796	1.19	8.83	0.82	0.054	0.86
Between occasions × sexes	3	743	1.11	1.23	0.12	0.005	0.08
Between occasions × subjects in sexes	30	668		10.71		0.062	
Total	47						

* Significant at better than the 5 percent level.

Blood samples were drawn from each subject on four occasions: (i) before hypnosis, (ii) during hypnosis, (iii) during the hypnotically induced anxiety state, and (iv) after hypnosis. Serum obtained from these samples was analyzed immediately for the delay in oxidation of N,N-dimethyl-*p*-phenylenediamine (lag time of Akerfeldt test) (*3*), the slope of the oxidation curve, and the concentration of ascorbic acid in the serum. The lag time and slope were measured as follows: 1.5 ml of 0.1 percent N,N-dimethyl-*p*-phenylenediamine dihydrochloride was added to 1.5 ml of serum, and the optical density at 552 mμ was determined in a Beckman DU spectrophotometer at 10-second intervals until oxidation of the substrate was complete. Serum and water (1.5 ml each) were employed as the blank. The lag time was taken as the time in seconds before oxidation of the substrate commenced and was obtained graphically from the intersection of the two rate curves of the over-all reaction. As the index of ceruloplasmin concentration, the average slope of the substrate oxidation curve was chosen (change in optical density per second). Serum ascorbic acid was determined by the method of Mindlin and Butler (*4*). Aprison and Grosz (*5*) have shown that the lag time is proportional to the ascorbic acid content of the serum.

The results of our experiment are given in Table 1. An analysis of variance for each variable by sex and occasion is given in Table 2. The female subjects exhibited a longer lag time, a greater slope, and a larger ascorbic acid level on each experimental occasion, but this tendency was significant only for the level of ascorbic acid. Although the lag time for females is almost double that for males, the enormous variability of individual response prevents this difference from attaining the conventional 5 percent confidence level. The female subjects also manifest higher ceruloplasmin levels on every testing occasion, but here again statistical significance is not achieved. The greater ascorbic acid level of the female subjects is most likely due to the much greater ascorbic acid intake by these subjects. The elevated ascorbic

acid levels of the female subjects are responsible for the longer lag times of these same individuals.

The effects of the various experimental manipulations on each variable measured are not significant. Despite a considerable increase in anxiety during the hypnosis plus anxiety occasion, the ceruloplasmin level did not change significantly. The anxiety levels achieved resembled those seen in severely disturbed, hospitalized psychiatric patients. Plasma hydrocortisone levels determined on aliquots of the blood samples drawn from the female subjects in this study were 75 percent higher during the anxiety occasion than during the hypnosis occasion (*2*).

The differences in conclusions between Ostfeld and his co-workers and ourselves concerning the effect of anxiety on serum ceruloplasmin level is not presently clear. They may reflect fundamental differences in the kind of anxiety studied, in the assessment of anxiety, or perhaps in differential effects resulting from chronic as contrasted with acute anxiety.

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Hybridization of Salmonella Species by Mating with Escherichia coli

Abstract. A number of species of *Salmonella* were fertile at low frequency as recipients in crosses with *Escherichia coli,* as evidenced by the isolation of lactose-positive hybrids possessing the *Salmonella* antigenic structure. A hybrid from an initial mating, when crossed again with *E. coli,* behaved then as a high-frequency recipient strain.

Genetic recombination in *Escherichia coli,* discovered by Tatum and Lederberg (*1*), and the fertility system involved in the mating between various strains of this species have been described in much detail (*2*). It is now apparent that a unilateral contribution of genetic material from the donor (Hfr, F^+) strain to the recipient (F^-, F^+) takes place. Recently, Luria and Burrous (*3*) have reported recombination between *E. coli* and many *Shigella* species which acted as F^- strains in these matings. Attempted crosses between *E. coli* and *Salmonella,* however, were unsuccessful until Baron *et al.* (*4*) detected recombination at low frequency between *E. coli* and *Salmonella typhimurium* strain TM-9. Subsequently, a streptomycin-resistant mutant of TM-9 was isolated prior to mating, and this strain (TM-9S^r-2) acted as a high-frequency recipient strain in matings with *E. coli.*

As an extension to the studies with *E. coli* K-12, Lederberg (*5*), using an appropriate screening procedure, surveyed a large number of other strains of *E. coli* for genetic recombination. In a similar study, following the initial observations with *S. typhimurium,* we have examined other species of *Salmonella* for ability to act as recipients in crosses with *E. coli* K-12, employing the following procedure.

The various *Salmonella* cultures were grown on nutrient agar plates, harvested after 18 hours' growth, and washed three times with saline. The donor culture of *E. coli* used was the strain of high frequency of recombination (Hfr) for lactose, isolated by Cavalli (*6, 7*). This culture was grown in Penassay broth, washed, and plated with each of the *Salmonella* cultures at a ratio of one Hfr (donor) cell of *E. coli* to 20 *Salmonella* (potential recipient) cells on minimal lactose agar plates. This medium would not support the growth of the parent methionine-requiring (M^-) *E. coli* Hfr cells or the parent lactose-negative (Lac^-) *Salmonella* species in control platings of the parent cultures alone, but would reveal methionine-independent lactose-positive (M^+Lac^+) progeny.

The results of this study have revealed that, in addition to the three strains of *S. typhimurium* (TM-9, LT-7, and HB) previously reported to be fertile by Baron, Carey, and Spilman (*8*), five strains of *S. typhosa,* two strains of *S. paratyphi* B, the Vi-containing East Africa strain of *S. paratyphi* C, *S. abortus-equi,* and *S. strasbourg* also gave rise to Lac^+ progeny which exhibited the antigenic characteristics of the parent *Salmonella* cultures. These strains and their antigenic structures, according to the Kauffmann-White schema (*9*), are listed in Table 1.

Table 1. *Salmonella* species yielding Lac^+ recombinants in crosses with *E. coli* Hfr.

Species	Antigenic structure	Recombination frequency	Source
S. typhimurium TM-9	4,5,12:i,1,2	4×10^{-8}	P. R. Edwards
S. typhimurium TM-9S^r-2	4,5,12:i,1,2	1×10^{-4}	From TM-9
S. typhimurium LT-7	1,4,5,12:i,1,2	1×10^{-7}	E. Englesberg
S. typhimurium HB	1,4,5,12:i,1,2	$< 10^{-8}$	Mouse
S. paratyphi B3	4,5,12:b,1,2	$< 10^{-8}$	P. R. Edwards
S. paratyphi B7	4,12:b,1,2	$< 10^{-8}$	P. R. Edwards
S. abortus-equi 26	4,12:_, enx	$< 10^{-8}$	P. R. Edwards
S. paratyphi C32	Vi 6,7:c,1,5	1×10^{-7}	P. R. Edwards
S. typhosa Ty2	Vi 9,12:d	$< 10^{-8}$	A. Felix
S. typhosa Ty2W	_9,12:d	$< 10^{-8}$	From Ty2
S. typhosa H-901	_9,12:d	$< 10^{-8}$	A. Felix
S. typhosa 0-901	_9,12:_	$< 10^{-8}$	A. Felix
S. typhosa 643	Vi 9,12:d	$< 10^{-8}$	A. Wolff
S. typhosa 643L$^-$	Vi 9,12:d	1×10^{-4}	From 643L$^+$
S. strasbourg 148	9:d,1,7	$< 10^{-8}$	P. R. Edwards

No recombinants were obtained from approximately 70 other species or strains of *Salmonella* with the same or similar serotypes, although in some instances heavy background growth, probably due to reciprocal feeding of the mixed cultures, may have obscured the results. In any case, there appears to be no evidence for any association between known antigenic components in the *Salmonella* tested and their ability to act as recipients in these crosses.

As a consequence of these and earlier experiments (*4*), it was assumed that populations of *Salmonella* cells generally are unable to act as recipients. For the sake of clarity, these cultures will be referred to as F^0 strains. At a low frequency, the F^0 cells in the population of certain or, perhaps, many species of *Salmonella* mutate to or in some manner acquire the F^- recipient state. These occasional F^- cells in the F^0 population will mate with the Hfr *E. coli.* A hybrid which is isolated from an initial mating (*E. coli* Hfr × *Salmonella*) should, according to this concept, be F^- and, hence, capable of being mated again as a recipient strain to yield hybrids at a high frequency. Recombination of such a strain should be detected also with the lower-frequency donor *E. coli* F^+ strains. The availability of a number of selective markers in *S. typhosa* strain 643 enabled us to test this possibility (*10*). A Lac^+ hybrid of strain 643 was selected for further examination, since it still possessed the antigenic and biochemical manifestations (other than