TABLE III. Corticosterone Levels in Adrenal Effluent after Repeated Bleedings and ACTH.

Animal		μg corticosterone/100 ml plasma at 0 time	30 min.	60 min.	90 min.
Pheasants					
No treatment	(3)*	25.7 ± 5.5†	34.0 ± 12.3†	31.0 ± 3.7†	
8 IU ACTH IV	(4)	29.6 ± 5.4	84.6 ± 34.5	104.6 (2)	
Chickens					
No treatment	(3)	27.9 ± 7.2	56.8 ± 15.3		26.3 ± 12.0
8 IU ACTH IV	(3)	30.7 ± 3.6	68.3 ± 14.5	143.7 ± 50.7	

* No. of animals. † Mean ± S.D.

creases of 123% and 368% at these same times. Urist and Deutsch(5), using the method of Silber, report an 85% increase in corticosterone level of peripheral plasma of male fowl following administration of 400 I.U. of ACTH. Phillips and Jones(2) found a decrease in amount of corticosterone in adrenal effluent blood of the capon following ACTH treatment.

Summary. A method is described for obtaining samples of adrenal venous blood from the bird. This allows avian species to be used for routine *in vivo* studies designed to assess adrenal activity by direct measurement. Levels of corticosterone, expressed as μg %, in adrenal effluent and peripheral plasma were 42.4 and 7.8 in turkeys and 34.8 and 8.6 in pheasants. In domestic fowl the values were 44.3 and 7.3 in Wisco Whites; 48.8 and 12.3 in New Hampshires, and 37.0 and 9.5 in White Rocks. ACTH (8 I.U. intravenously) increased the output of corticosterone from 29.6 to 104.6 μg % in pheasants and from 30.7 to 143.7 μg % in chickens 60 minutes after injection.

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Comparative Bilirubin Levels in Vitreous Body, Synovial Fluid, Cerebrospinal Fluid and Serum after Death. (26012)

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Further studies on postmortem chemistry (7-11) revealed in cases of jaundice presence of bilirubin not only in cerebrospinal fluid, where it was known to occur(1-6), but also in vitreous body and synovial fluid. This to our knowledge has not been reported before, and it seemed of interest, therefore, to obtain data on comparative concentrations in these fluids.

Materials and methods. Material from autopsies about 5½ hours after death on the average was based on a total of 78 male adult bodies including 44 cases of jaundice and 34 non-jaundice control cases. Spinal fluid was obtained by cisternal puncture(7,9,10), vitreous body by puncture of the eye balls at the outer canthus with a 20 gauge needle and 10 cc syringe(11), and synovial fluid was aspirated from the knee joints with a 16 gauge needle and 10 cc syringe. Bilirubin determinations were performed by the method of Malloy and Evelyn(13) with the usual 1 minute modification for direct bilirubin(13). Floccules of mucoprotein forming in dilute syno-

TABLE I. Comparative Averages and Ranges of Bilirubin Levels in Postmortem Vitreous Body, Cerebrospinal Fluid, Synovial Fluid and Serum.

	Values in jaundice			Postmortem normals			Antemortem normals	
		Bilirubin			Bilirubin		Bilirubin	
Body fluids	No. of cases	Total	Direct	No. of cases	Total	Direct	Total	Direct
		mg/100 ml			mg/100 ml			
Vitreous body	43	.04 ± .02 0 – .48	.01 ± .02 0 – .29	29	0	0	no data	
Spinal fluid	43	.23 ± .07 0 – 4.00	.11 ± .06 0 – 2.47	34	0	0	0	0
Synovial fluid	33	2.02 ± .31 0 – 10.5	1.11 ± .55 0 – 5.50	12	.28 ± .07 .15 – .75	.1 ± .04 0 – .48	no data	
Serum	43	8.6 ± .27 1.51 – 36.0	4.8 ± .34 .19 – 24.3	12	.9 ± .16[6] .2 – 1.5	.4 ± .09[6] .1 – .8	.62 ± .25[13] .2 – .8[8]	.11 ± .05[13] .1 – .2[8]

± indicates stand. dev. of mean.

vial fluid on acidification with diazo or blank reagent were resuspended by short vigorous shaking. The resulting bubbles usually rose rapidly enough to allow colorimetry after one minute.

Results. Table I summarizes averages and ranges of bilirubin concentrations in vitreous body, spinal fluid, synovial fluid and serum found in jaundice with bilirubin levels of more than 1.5 mg/100 ml and in normal non-jaundice cases with bilirubin levels of 1.5 mg/100 ml or less. Averages of total bilirubin in the present series of jaundice cases were 8.6 mg/100 ml in serum, 2.02 in synovial fluid, 0.23 in spinal fluid and 0.04 in vitreous body. As seen from the ranges, highest values of total bilirubin encountered in jaundice were 36.0 mg/100 ml in serum, 10.5 in synovial fluid, 4.0 in spinal fluid and 0.48 in vitreous. In normal non-jaundice cases free from hepatic, hemolytic and joint diseases and hemorrhagic contaminations bilirubin was generally absent in spinal fluid and vitreous but present in synovial fluid averaging 0.28 mg/100 ml for total bilirubin. This compared to normal serum values of 0.9 mg postmortem and 0.62 mg/100 ml antemortem. No data on normal antemortem synovial bilirubin were found in the literature nor statements on the presumed absence of pigment in the vitreous.

Ratios of bilirubin averages in vitreous, spinal and synovial fluid to serum bilirubin averages of the present jaundice cases (Table I) are presented in Table II. Total bilirubin values in the vitreous were 220 times smaller, in spinal fluid 35 times and in synovial fluid 4 times smaller than in serum. The direct bilirubin in vitreous was 480 times smaller than in serum, while the other direct bilirubin ratios approached or matched those of total bilirubin. By applying these ratios when serum bilirubin is known one may obtain an idea of what level could be expected in any of the other fluids. For instance, assuming a total serum bilirubin of 20 mg/100 ml, the expected bilirubin level in the vitreous would be 20/220 or about 0.1 mg, in spinal fluid 20/35 or about 0.5 mg and in synovial fluid 20/4 or about 5 mg/100 ml. Again, if 0.05 mg/100 ml were considered the limit of detectable bilirubin such traces could be expected in synovial fluid, spinal fluid and vitreous when serum bilirubin levels were about 0.05 x 4 = 0.2 mg, 0.05 x 35 = 1.8 mg and 0.05 x 220 = 11 mg/100 ml respectively.

It is obvious that the ratios given in Table II can not be more than approximations, and not infrequently exceptions from the rule will be found. In the present series traces of bilirubin have sometimes been observed in vitreous and spinal fluid when serum total bilirubin was less than 0.5 mg/100 ml. On the other hand, bilirubin was absent in vitreous and spinal fluid when total serum bilirubin

TABLE II. Ratios of Bilirubin Averages in Vitreous Body, Spinal and Synovial Fluid to Average Serum Bilirubin.

Bilirubin ratios of	Total bilirubin	Direct bilirubin
Vitreous body : Serum	1 : 220	1 : 480
Spinal fluid : Serum	1 : 35	1 : 45
Synovial fluid : Serum	1 : 4	1 : 4

was as high as 18.7 mg/100 ml. However, in general, bilirubin ratios will approach those given above.

Discussion. Occurrence of bilirubin in spinal fluid of the newborn(6), in jaundice (1-4,10) and after hemorrhages(2,4) has been known for some time. However, there seem to be no data in the literature about presence of bilirubin in the vitreous, although xanthochromia of the aqueous humor is mentioned by Krause(3). Yet there is evidence that its effect may have been noted clinically by observation of xanthopsia, yellow vision, which may occur in jaundice as well as santonin poisoning(12). The mechanism of xanthopsia in jaundice could be explained as an optic filter effect due to yellow coloration of the vitreous body and probably the other visual media.

No reports on bilirubin in synovial fluid were encountered except for contribution on use of bilirubin determinations for diagnosing a traumatic origin of joint effusions(14). In this connection it may be of interest that our material also included 4 cases in which synovial bilirubin was higher than serum bilirubin suggesting intraarticular production of part of the bilirubin from hemoglobin. In contrast to vitreous and spinal fluid there were high values of synovial bilirubin in jaundice, and non-jaundice cases showed it with sufficient frequency to conclude that bilirubin is a normal constituent of synovial fluid.

Ratios between total bilirubin of the fluids given in Table II indicate a diffusion gradient from vitreous to spinal fluid to synovial fluid. This effect may be due partly to the difference in surface of the diffusion membrane, which is smallest in the globular periphery of vitreous, much larger in choroid plexus and probably considerable in synovial villi.

Regarding the validity of postmortem chemistry for *intra vitam* application, bilirubin besides calcium has been found to be one of the stable substances after death(8,9). Since the ranges of normal postmortem and antemortem total and direct serum bilirubin are close, it may safely be assumed that the given postmortem bilirubin values in vitreous and synovial fluid will also approach closely those during life.

Summary. A total of 78 autopsies including 44 jaundice and 34 non-jaundice control cases have been studied for comparative bilirubin levels in vitreous body, spinal fluid, synovial fluid and serum. Ratios of total bilirubin averages in vitreous, spinal and synovial fluid to average total serum bilirubin were 1:220, 1:35 and 1:4 respectively. Highest and average values of total bilirubin found were 0.48 and 0.04 mg/100 ml in vitreous body and 10.5 and 2.02 mg/100 ml in synovial fluid respectively. Total synovial bilirubin in non-jaundice cases averaged 0.28 mg/100 ml. Bilirubin may thus be considered a normal constituent of synovial fluid.

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